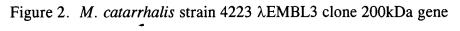
Subclones of portions of the 200 kDa protein gene from λ EMBL3 clone 8II and PCR amplification of 5' region

Sal pKS5 (4.9 kb) pKS59, pKS63 and pKS71 (1.4 kb) **%** pKS47 (1.1 kb) X λΕΜΒL3 clone 8II pKS9 and pKS10 (11 kb)

ATG GTG 200 kD protein gene

4211 4166 : amplified 0.7 kb



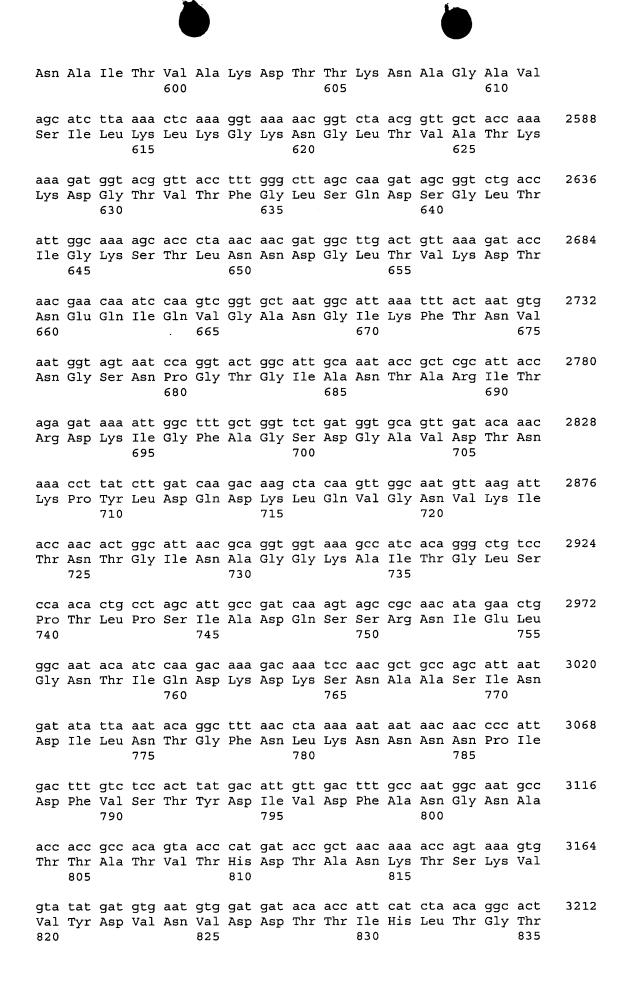


ccat	ggat	tat 9	gggca	aggt	gt g	ctcg	cctgo	c cgt	tatga	atgg	cgat	tgaca	acc	ccatt	tgccc	60
cata	tctg	gta (cgati	tgad	ca to	gtgat	tatga	a tti	taaca	atgt	gaca	atgai	ttt .	aacat	tgttt	120
aata	ctgt	tg (ccat	catta	ac ca	ataat	ttag	g taa	acgca	attt	agta	aacgo	cat	ttgta	aaaaat	180
catt	gcgo	ccc (cttta	atgt	gt ai	tcata	atgaa	a tag	gaata	atta	tgat	ttgta	atc	tgatt	attgt	240
atca	gaat	gg 1	tgate	gctat	a to	gatga	atgco	c tac	cgagt	tga	tttg	gggti	taa	tcact	ctatg	300
attt	gata	ata 1	tttt	gaaa	ct aa	atcta	attga	a cti	caaat	ccac	cata	atggi	tta	taatt	tagca	360
taat	ggta	agg (cttti	tgta	aa aa	aatca	acato	gc:	aatai	tgt	tcta	actg	tta ·	ctaco	catgct	420
tgaa	tgad	cga 1	tccca	aatca	ac ca	agatt	catt	caa	agtga	atgt	gtt	tgtai	tac (gcaco	cattta	480
ccct	aatt	at 1	ttcaa	atcaa	aa t	gccta	atgto	c ago	catg	tatc	att	tttt	taa (ggtaa	aaccac	540
catg	aato	cac a	atcta	ataaa	ag to	catct	ttaa	a caa	aagco	caca	ggca	acati	tta	tggca	agtggc	600
agag	tacg	gcc a	aaato	ccca	ca go	cacgo	gggg	gg(ggtag	gctg	tgct	tacag	ggg	caagt	tggca	660
gtgt	atgo	cac 1	tctga	agctt	t go	cccgt	atto	g cc	geget	cgc	tgt	cctc		atc Ile		716
_	_		_		_									aaa Lys		764
														gcc Ala		812
														gca Ala 50		860
ggc Gly	ggt Gly	caa Gln	gcc Ala 55	atc Ile	gcc Ala	atc Ile	ggt Gly	agt Ser 60	agt Ser	aat Asn	aaa Lys	act Thr	gtc Val 65	aat Asn	gga Gly	908
														tcc Ser		956
														gcc Ala		1004
														aaa Lys		1052
														gta Val 130		1100

	_		_	_	tca Ser	_	_		_	_						1148
					gcc Ala											1196
_					aac Asn	_					_		_		_	1244
					gtg Val 185											1292
					tct Ser											1340
					act Thr											1388
				_	gtc Val											1436
					cag Gln											1484
					cca Pro 265											1532
					ggt Gly				Asn	Lys		Asp				1580
					gcg Ala											1628
					gat Asp											1676
					aaa Lys											1724
aat Asn 340	aat Asn	atc Ile	ggt Gly	gtg Val	gta Val 345	aaa Lys	gag Glu	gct Ala	gat Asp	aat Asn 350	agt Ser	ggt Gly	ctg Leu	aaa Lys	gtt Val 355	1772
					tta Leu											1820

360 365 370

					aca Thr											1868
					gat Asp											1916
_		_		_	aaa Lys		_			_				_		1964
			_	_	aca Thr 425		_	_					_			2012
_	_				ttt Phe	-	-	_								2060
_			_	_	aaa Lys							_	_	_		2108
		_			att Ile	_	_				_					2156
_			_	_	gct Ala		-		_			_	_			2204
_	_	_			tta Leu 505		_		_			_	_			2252
	_			_	gat Asp	_	_	_			_		_			2300
					aaa Lys											2348
gat Asp					aag Lys											2396
					agc Ser											2444
					ttt Phe 585											2492
aac	gct	atc	acc	gtg	gct	aaa	gat	acg	aca	aaa	aat	gcc	ggc	gca	gtc	2540



													aac Asn			3260
_	_					_					_		tct Ser 865			3308
_	_	_		_		_		_		_	_		cta Leu			3356
													acc Thr			3404
				-		_	_	_	_				gct Ala	_	_	3452
													caa Gln			3500
						_							acc Thr 945			3548
													ctt Leu			3596
													aac Asn			3644
	Ser					Val				Gly		Lys	ttt Phe			3692
			Asn					Āla					aca Thr			3740
		Arg					Phe					Gly	tca Ser 1025			3788
	Ser					Ser					Asn		ggt Gly			3836
Lys	att Ile 1045	acc Thr	aac Asn	att Ile	Gln	tca Ser 1050	ggt Gly	gag Glu	att Ile	Ala	caa Gln 1055	aac Asn	agc Ser	cat His	gat Asp	3884
gct Ala 1060	Val	aca Thr	ggc Gly	Gly	aag Lys 1065	att Ile	tat Tyr	gat Asp	Leu	aaa Lys 1070	acc Thr	gaa Glu	ctt Leu	Glu	aac Asn 1075	3932

aaa atc agc agt act gcc aaa aca gca caa aac tca tta cac gaa ttc Lys Ile Ser Ser Thr Ala Lys Thr Ala Gln Asn Ser Leu His Glu Phe 1080 1085 1090	3980
tca gta gca gat gaa caa ggt aat aac ttt acg gtt agt aac cct tac Ser Val Ala Asp Glu Gln Gly Asn Asn Phe Thr Val Ser Asn Pro Tyr	4028
tcc agt tat gac acc tca aag acc tct gat gtc atc acc ttt gca ggt Ser Ser Tyr Asp Thr Ser Lys Thr Ser Asp Val Ile Thr Phe Ala Gly 1110 1115 1120	4076
gaa aac ggc att acc acc aag gta aat aaa ggt gtg gtg cgt gtg ggc Glu Asn Gly Ile Thr Thr Lys Val Asn Lys Gly Val Val Arg Val Gly 1125 1130 1135	4124
att gac caa acc aaa ggc tta acc acg cct aag ctg acc gtg ggt aat Ile Asp Gln Thr Lys Gly Leu Thr Thr Pro Lys Leu Thr Val Gly Asn 1140 1145 1150 1155	4172
aat aat ggc aaa ggc att gtc att gac agc caa aat ggt caa aat acc Asn Asn Gly Lys Gly Ile Val Ile Asp Ser Gln Asn Gly Gln Asn Thr 1160 1165 1170	4220
atc aca gga cta agc aac act cta gct aat gtt acc aat gat aaa ggt Ile Thr Gly Leu Ser Asn Thr Leu Ala Asn Val Thr Asn Asp Lys Gly 1175 1180 1185	4268
agc gta cgc acc aca gaa cag ggc aat ata atc aaa gac gaa gac aaa Ser Val Arg Thr Thr Glu Gln Gly Asn Ile Ile Lys Asp Glu Asp Lys 1190 1195 1200	4316
acc cgt gcc gcc agc att gtt gat gtg cta agc gca ggc ttt aac ttg Thr Arg Ala Ala Ser Ile Val Asp Val Leu Ser Ala Gly Phe Asn Leu 1205 1210 1215	4364
caa ggc aat ggt gaa gcg gtt gac ttt gtc tcc act tat gac acc gtc Gln Gly Asn Gly Glu Ala Val Asp Phe Val Ser Thr Tyr Asp Thr Val 1220 1225 1230 1235	4412
aac ttt gcc gat ggc aat gcc acc gct aag gtg acc tat gat gac Asn Phe Ala Asp Gly Asn Ala Thr Thr Ala Lys Val Thr Tyr Asp Asp 1240 1245 1250	4460
aca agc aaa acc agt aaa gtg gtc tat gat gtc aat gtg gat gat aca Thr Ser Lys Thr Ser Lys Val Val Tyr Asp Val Asn Val Asp Asp Thr 1255 1260 1265	4508
acc att gaa gtt aaa gat aaa aaa ctt ggc gta aaa acc acc aca ttg Thr Ile Glu Val Lys Asp Lys Lys Leu Gly Val Lys Thr Thr Thr Leu 1270 1275 1280	4556
acc agt act ggc aca ggt gct aat aaa ttt gcc cta agc aat caa gct Thr Ser Thr Gly Thr Gly Ala Asn Lys Phe Ala Leu Ser Asn Gln Ala 1285 1290 1295	4604
act ggc gat gcg ctt gtc aag gcc agt gat atc gtt gct cat cta aac Thr Gly Asp Ala Leu Val Lys Ala Ser Asp Ile Val Ala His Leu Asn	4652

1300	1305	1310	1315
Thr Leu Ser Gly	-	act gcc aaa ggg gca Thr Ala Lys Gly Ala 1325	_
		gct gat ggc aat aag Ala Asp Gly Asn Lys 1340	
	Lys Tyr Tyr (caa gcc aaa aat gat Gln Ala Lys Asn Asp 355	
		gac aaa ctg gtc gcc Asp Lys Leu Val Ala 1375	
		atg aat gtc aaa tca Met Asn Val Lys Ser 1390	
Glu Gln Val Asn	-	aaa aag caa ggc atc Lys Lys Gln Gly Ile 1405	
		aaa gcc gct tct gat Lys Ala Ala Ser Asp 1420	
	Thr Val Gly	gat tta aat gcc gtt Asp Leu Asn Ala Val 435	
		ggc aca acg gct aaa Gly Thr Thr Ala Lys 1455	
		caa aca gac acc aat Gln Thr Asp Thr Asn 1470	
Asn Asn Ile Gly		ggt act gat ggc ttc Gly Thr Asp Gly Phe 1485	
		aac agc gtt aat gca Asn Ser Val Asn Ala 1500	
	Gly Val Ser	ttt gta gac tca agc Phe Val Asp Ser Ser 515	
		gcc aat ggg ctg gac Ala Asn Gly Leu Asp 1535	
gtc atc agt aat	gtg ggc aaa g	ggc aca aaa gat acc	gac gct gcc aat 5372

	Gly Lys Gly Thr 1545	Lys Asp Thr Asp Ala Ala Ass 1550 1559	
	Glu Val Arg Asn	ttg ttg ggt ctt ggt aat gct Leu Leu Gly Leu Gly Asn Ala .565 1570	
		gta aac att gcc gac atc aaa Val Asn Ile Ala Asp Ile Lys 1585	
		aac cgc act gtc atc aaa gca Asn Arg Thr Val Ile Lys Ala 1600	
		aac gat acc gaa aaa ctt gco Asn Asp Thr Glu Lys Leu Ala 1615	
Thr Gly Gly Ile Gln		aaa gac ggc aac gct aac ggc Lys Asp Gly Asn Ala Asn Gly 1630	7
	Trp Val Lys Thr	caa aaa gat ggc agc aaa aaa Gln Lys Asp Gly Ser Lys Lys .645 1650	
		ggt cag acc aac tat ttg acc Gly Gln Thr Asn Tyr Leu Thi 1665	
		ata aat gaa caa ggt atc cgo Ile Asn Glu Gln Gly Ile Arg 1680	
		gag cct gtg gta caa ggg cgt Glu Pro Val Val Gln Gly Arg 1695	
Asn Gly Ile Asp Ser		aag cac tca gtg gcg ata ggt Lys His Ser Val Ala Ile Gly 1710 1715	7
	Asp Gly Glu Ala	gcc gtt gcc ata ggc aga caa Ala Val Ala Ile Gly Arg Glr 725 1730	
		atc ggt gat aac gca caa gco Ile Gly Asp Asn Ala Gln Ala 1745	
		aca ggc aat gtg gta gca ggt Thr Gly Asn Val Val Ala Gly 1760	
		agc act gtt aag gct gat aad Ser Thr Val Lys Ala Asp Asr 1775	

agt tac agt gtg ggt aat aac aac cag ttt acc gat gcc act caa acc 6 Ser Tyr Ser Val Gly Asn Asn Asn Gln Phe Thr Asp Ala Thr Gln Thr 1780 1785 1790 1795	092
gat gtc ttt ggt gtg ggc aat aac atc acc gtg acc gaa agt aac tcg 6 Asp Val Phe Gly Val Gly Asn Asn Ile Thr Val Thr Glu Ser Asn Ser 1800 1805 1810	140
gtt gcc tta ggt tca aac tct gcc atc agt gca ggc aca cac gca ggc 6 Val Ala Leu Gly Ser Asn Ser Ala Ile Ser Ala Gly Thr His Ala Gly 1815 1820 1825	188
aca caa gcc aaa aaa tct gac ggc aca gca ggt aca acc acc aca gca 6 Thr Gln Ala Lys Lys Ser Asp Gly Thr Ala Gly Thr Thr Thr Thr Ala 1830 1835 1840	236
ggt gca acc ggt acg gtt aaa ggc ttt gct gga caa acg gcg gtt ggt 6 Gly Ala Thr Gly Thr Val Lys Gly Phe Ala Gly Gln Thr Ala Val Gly 1845 1850 1855	284
gcg gtc tcc gtg ggt gcc tca ggt gct gaa cgc cgt atc caa aat gtg 6 Ala Val Ser Val Gly Ala Ser Gly Ala Glu Arg Arg Ile Gln Asn Val 1860 1865 1870 1875	332
gca gca ggt gag gtc agt gcc acc agc acc gat gcg gtc aat ggt agc 6 Ala Ala Gly Glu Val Ser Ala Thr Ser Thr Asp Ala Val Asn Gly Ser 1880 1885 1890	380
cag ttg tac aaa gcc acc caa agc att gcc aac gca acc aat gag ctt 6 Gln Leu Tyr Lys Ala Thr Gln Ser Ile Ala Asn Ala Thr Asn Glu Leu 1895 1900 1905	428
gac cat cgt atc cac caa aac gaa aat aag gcc aat gca ggg att tca 6 Asp His Arg Ile His Gln Asn Glu Asn Lys Ala Asn Ala Gly Ile Ser 1910 1915 1920	476
tca gcg atg gcg atg gcg tcc atg cca caa gcc tac att cct ggc aga 6 Ser Ala Met Ala Met Ala Ser Met Pro Gln Ala Tyr Ile Pro Gly Arg 1925 1930 1935	524
tcc atg gtt acc ggg ggt att gcc acc cac aac ggt caa ggt gcg gtg 6 Ser Met Val Thr Gly Gly Ile Ala Thr His Asn Gly Gln Gly Ala Val 1940 1945 1950 1955	572
gca gtg gga ctg tcg aag ctg tcg gat aat ggt caa tgg gta ttt aaa 6 Ala Val Gly Leu Ser Lys Leu Ser Asp Asn Gly Gln Trp Val Phe Lys 1960 1965 1970	620
atc aat ggt tca gcc gat acc caa ggc cat gta ggg gcg gca gtt ggt 6 Ile Asn Gly Ser Ala Asp Thr Gln Gly His Val Gly Ala Ala Val Gly 1975 1980 1985	668
gca ggt ttt cac ttt taagccataa atcgcaagat tttacttaaa aatcaatctc 6 Ala Gly Phe His Phe 1990	723
accatagttg tataaaacag catcagcatc agtcatatta ctgatgctga tgtttttat 6	783
cacttaaacc attttaccgc tcaagtgatt ctctttcacc atgaccaaat cgccattgat 6	843

cataggtaaa	cttattgagt	aaattttatc	aatgtagttg	ttagatatgg	ttaaaattgt	6903
gccattgacc	aaaaaatgac	cgatttatcc	cgaaaatttc	tgattatgat	ccgttgacct	6963
gcaggtcgac						6973

Figure 3. M. catarrhalis strain 4223 genomic 200kDa gene.

ccatggatat gggcaggtgt	getegeetge egt	tatgatgg cga	gacacc ccat	tgccc 60
catatetgta egatttgaca	tgtgatatga tt	taacatgt gaca	atgattt aaca	ttgttt 120
aatactgttg ccatcattac	cataatttag taa	acgcattt agta	aacgcat ttgta	aaaaat 180
cattgcgccc ctttatgtgt	atcatatgaa tag	gaatatta tga	tgtatc tgat	tattgt 240
atcagaatgg tgatgctata	tgatgatgcc tac	cgagttga ttt	gggttaa tcac	tctatg 300
atttgatata ttttgaaact	aatctattga ct	taaatcac cata	atggtta taat	ttagca 360
taatggtagg ctttttgtaa	aaatcacatc gca	aatattgt tcta	actgtta ctace	catgct 420
tgaatgacga tcccaatcac	cagattcatt caa	agtgatgt gtt	tgtatac gcac	cattta 480
ccctaattat ttcaatcaaa	tgcctatgtc ago	catgtatc att	ttttaa ggta	aaccac 540
c atg aat cac atc tat Met Asn His Ile Tyr 1 5	Lys Val Ile Pl		la Thr Gly T	
atg gca gtg gca gag t Met Ala Val Ala Glu T 20				
tgt gct aca ggg caa g Cys Ala Thr Gly Gln V 35				
att gcc gcg ctc gct g Ile Ala Ala Leu Ala V 50				
gct tat gct caa aaa a Ala Tyr Ala Gln Lys L 65				
aac cag cca aga cgc t Asn Gln Pro Arg Arg S 85				
att gct att ggt gaa a Ile Ala Ile Gly Glu A 100				
atc ggt agt agt aat a Ile Gly Ser Ser Asn L 115				
ggt acc gat gct acg g Gly Thr Asp Ala Thr G 130	ggt caa gag tcc ly Gln Glu Ser 135	atc gcc atc Ile Ala Ile 140	ggt ggt gat Gly Gly Asp	gta 973 Val
aag gct agt ggt gat g Lys Ala Ser Gly Asp A				

145					150					155					160	
														ctg Leu 175		1069
	_						-	-			_		_	agc Ser		1117
														cac His		1165
														tcc Ser		1213
														gca Ala		1261
														ggt Gly 255		1309
														ggt Gly		1357
_		_		_		_		-						gtt Val		1405
														acc Thr		1453
														ggt Gly		1501
														gtc Val 335		1549
														gaa Glu		1597
														gat Asp		1645
														att Ile		1693
ggt	ggt	gca	gag	acc	aac	gca	tta	acc	gat	aat	aat	atc	ggt	gtg	gta	1741

Gly 385	Gly	Ala	Glu	Thr	Asn 390	Ala	Leu	Thr	Asp	Asn 395	Asn	Ile	Gly	Val	Val 400	
		_	_	aat Asn 405	_		_		_			_				1789
				gag Glu												1837
_	_	_		agt Ser												1885
				acc Thr												1933
				gtt Val												1981
	-	_		ggc Gly 485			_			-	_					2029
-		-		gat Asp	_	_	_			_						2077
				gtg Val												2125
_	_			aaa Lys	_		_			_			_	_	_	2173
				acc Thr												2221
				ggc Gly 565												2269
_	_	_		aat Asn	_		_									2317
				aac Asn												2365
				aac Asn												2413

				aat Asn 630									2461
	_	_	_	gac Asp	_	_	_	_		_		_	2509
				aat Asn									2557
				acg Thr									2605
				gat Asp									2653
				act Thr 710									2701
				aaa Lys									2749
				acc Thr									2797
				gca Ala									2845
				ggc Gly		Val			Thr		Thr		2893
				atc Ile 790									2941
_	_	_	_	cgc Arg			_	_					2989
				gct Ala									3037
				aat Asn									3085
				gcc Ala									3133

cat gat acc His Asp Thi 865					Tyr Asp		
gat gat aca Asp Asp Th		His Leu		_	_		
ggc gtc aaa Gly Val Lys		_		Thr Ser	_		
gca act aad Ala Thr Asi 91!	n Phe Asn						
gcc aaa gad Ala Lys Asp 930	_	_				_	
acc acc aaa Thr Thr Lys 945			_			_	
aag gta gat Lys Val Asp							
ggt caa aag Gly Gln Lys	-						
gaa aac ggt Glu Asn Gly 999	Leu Asn	Ile Lys	_			_	
ggc att aad Gly Ile Asi 1010		Ser Gly	Leu Lys	Ala Gly	Lys Ser		
gac ggt ggc Asp Gly Gly 1025	Leu Ser					Gln Ile	
gtc ggt gct Val Gly Ala			Phe Ala				
gta ggt gct Val Gly Ala					Thr Arg		
ggc ttt act Gly Phe Thi 1075	Gly Thr	Asn Gly					
agc aaa gad Ser Lys Asp							

1090 1095 1100

tca ggt gag att gcc caa aac agc cat gat gct gtg aca ggc ggc ag Ser Gly Glu Ile Ala Gln Asn Ser His Asp Ala Val Thr Gly Gly Ly 1105 1110 1115	/s
att tat gat tta aaa acc gaa ctt gaa aac aaa atc agc agt act go Ile Tyr Asp Leu Lys Thr Glu Leu Glu Asn Lys Ile Ser Ser Thr A 1125 1130 1135	
aaa aca gca caa aac tca tta cac gaa ttc tca gta gca gat gaa ca Lys Thr Ala Gln Asn Ser Leu His Glu Phe Ser Val Ala Asp Glu G 1140 1145 1150	
ggt aat aac ttt acg gtt agt aac cct tac tcc agt tat gac acc tc Gly Asn Asn Phe Thr Val Ser Asn Pro Tyr Ser Ser Tyr Asp Thr Se 1155 1160 1165	
aag acc tct gat gtc atc acc ttt gca ggt gaa aac ggc att acc ac Lys Thr Ser Asp Val Ile Thr Phe Ala Gly Glu Asn Gly Ile Thr Th 1170 1175 1180	
aag gta aat aaa ggt gtg gtg cgt gtg ggc att gac caa acc aaa gg Lys Val Asn Lys Gly Val Val Arg Val Gly Ile Asp Gln Thr Lys G 1185 1190 1195 120	ly
tta acc acg cct aag ctg acc gtg ggt aat aat aat ggc aaa ggc at Leu Thr Thr Pro Lys Leu Thr Val Gly Asn Asn Asn Gly Lys Gly I 1205 1210 1215	
gtc att gac agc caa aat ggt caa aat acc atc aca gga cta agc ag Val Ile Asp Ser Gln Asn Gly Gln Asn Thr Ile Thr Gly Leu Ser As 1220 1225 1230	
act cta gct aat gtt acc aat gat aaa ggt agc gta cgc acc aca ga Thr Leu Ala Asn Val Thr Asn Asp Lys Gly Ser Val Arg Thr Thr G 1235 1240 1245	
cag ggc aat ata atc aaa gac gaa gac aaa acc cgt gcc gcc agc at Gln Gly Asn Ile Ile Lys Asp Glu Asp Lys Thr Arg Ala Ala Ser I 1250 1255 1260	
gtt gat gtg cta agc gca ggc ttt aac ttg caa ggc aat ggt gaa gg Val Asp Val Leu Ser Ala Gly Phe Asn Leu Gln Gly Asn Gly Glu A 1265 1270 1275 128	la
gtt gac ttt gtc tcc act tat gac acc gtc aac ttt gcc gat ggc ac Val Asp Phe Val Ser Thr Tyr Asp Thr Val Asn Phe Ala Asp Gly As 1285 1290 1295	
gcc acc acc gct aag gtg acc tat gat gac aca agc aaa acc agt acc Ala Thr Thr Ala Lys Val Thr Tyr Asp Asp Thr Ser Lys Thr Ser Lys 1300 1305 1310	
gtg gtc tat gat gtc aat gtg gat gat aca acc att gaa gtt aaa ga Val Val Tyr Asp Val Asn Val Asp Asp Thr Thr Ile Glu Val Lys As 1315 1320 1325	
aaa aaa ctt ggc gta aaa acc acc aca ttg acc agt act ggc aca gg	gt 4573

Lys Lys Leu Gly Val :	Lys Thr Thr Thr Leu 1335	Thr Ser Thr Gly Thr G	Bly
Ala Asn Lys Phe Ala	Leu Ser Asn Gln Ala	act ggc gat gcg ctt g Thr Gly Asp Ala Leu V 1355 13	
		acc tta tct ggc gac a Thr Leu Ser Gly Asp I 1375	
		aac tca gca ggc tat g Asn Ser Ala Gly Tyr V 1390	_
		agt acc gat aac aag t Ser Thr Asp Asn Lys T 1405	
		aaa acc aaa gaa gtt g Lys Thr Lys Glu Val A 1420	
Lys Asp Lys Leu Val	Ala Gln Ala Gln Thr	cca gat ggc aca ttg g Pro Asp Gly Thr Leu A 1435 14	
	_	gaa caa gta aat gat g Glu Gln Val Asn Asp A 1455	
		gcc ttt gtt aaa gga c Ala Phe Val Lys Gly I 1470	
	_	aac gcc gca gta act g Asn Ala Ala Val Thr V 1485	
		ctg acc ttt gca ggg g Leu Thr Phe Ala Gly A 1500	
Thr Gly Thr Thr Ala	Lys Lys Leu Gly Glu	act ttg acc atc aaa g Thr Leu Thr Ile Lys G 1515 15	
		aat aac atc ggt gtg g Asn Asn Ile Gly Val V 1535	
		gcc aaa gac cta acc a Ala Lys Asp Leu Thr A 1550	
		att gat gac aaa ggc g Ile Asp Asp Lys Gly V 1565	

		gca aac acc cct gtg cta Ala Asn Thr Pro Val Leu 1580	5293
Ser Ala Asn Gly Leu	Asp Leu Gly Gly Lys	gtc atc agt aat gtg ggc Val Ile Ser Asn Val Gly 1595 1600	5341
		gta caa cag tta aac gaa Val Gln Gln Leu Asn Glu 1615	5389
		ggt aat gat aac gct gac Gly Asn Asp Asn Ala Asp 1630	5437
		aaa gac cca aat tca ggt Lys Asp Pro Asn Ser Gly 1645	5485
_	_	ggc acg gta ctt ggc ggt Gly Thr Val Leu Gly Gly 1660	5533
Lys Gly Asn Asn Asp	Thr Glu Lys Leu Ala	act ggt ggt ata caa gtg Thr Gly Gly Ile Gln Val 1675 1680	5581
		gat tta agc aat gtt tgg Asp Leu Ser Asn Val Trp 1695	5629
		gcc ctg ctc gcc act tat Ala Leu Leu Ala Thr Tyr 1710	5677
	-	aac aac ccc gca gaa gcc Asn Asn Pro Ala Glu Ala 1725	5725
		ttc ttc cat gtc aac gat Phe Phe His Val Asn Asp 1740	5773
Gly Asn Gln Glu Pro	Val Val Gln Gly Arg	aac ggc att gac tca agt Asn Gly Ile Asp Ser Ser 1755 1760	5821
		ttc cag gcc aag gca gat Phe Gln Ala Lys Ala Asp 1775	5869
		acc caa gca ggc aac caa Thr Gln Ala Gly Asn Gln 1790	5917
		acg ggc gat caa tcc atc Thr Gly Asp Gln Ser Ile 1805	5965

gcc atc ggt aca ggc aat gtg gta gca ggt aag cac tct ggt gcc atc Ala Ile Gly Thr Gly Asn Val Val Ala Gly Lys His Ser Gly Ala Ile 1810 1815 1820	6013
ggc gac cca agc act gtt aag gct gat aac agt tac agt gtg ggt aat Gly Asp Pro Ser Thr Val Lys Ala Asp Asn Ser Tyr Ser Val Gly Asn 1825 1830 1835 1840	6061
aac aac cag ttt acc gat gcc act caa acc gat gtc ttt ggt gtg ggc Asn Asn Gln Phe Thr Asp Ala Thr Gln Thr Asp Val Phe Gly Val Gly 1845 1850 1855	6109
aat aac atc acc gtg acc gaa agt aac tcg gtt gcc tta ggt tca aac Asn Asn Ile Thr Val Thr Glu Ser Asn Ser Val Ala Leu Gly Ser Asn 1860 1865 1870	6157
tct gcc atc agt gca ggc aca cac gca ggc aca caa gcc aaa aaa	6205
gac ggc aca gca ggt aca acc acc aca gca ggt gca acc ggt acg gtt Asp Gly Thr Ala Gly Thr Thr Thr Thr Ala Gly Ala Thr Gly Thr Val 1890 1895 1900	6253
aaa ggc ttt gct gga caa acg gcg gtt ggt gcg gtc tcc gtg ggt gcc Lys Gly Phe Ala Gly Gln Thr Ala Val Gly Ala Val Ser Val Gly Ala 1905 1910 1915 1920	6301
tca ggt gct gaa cgc cgt atc caa aat gtg gca gca ggt gag gtc agt Ser Gly Ala Glu Arg Arg Ile Gln Asn Val Ala Ala Gly Glu Val Ser 1925 1930 1935	6349
gcc acc agc acc gat gcg gtc aat ggt agc cag ttg tac aaa gcc acc Ala Thr Ser Thr Asp Ala Val Asn Gly Ser Gln Leu Tyr Lys Ala Thr 1940 1945 1950	6397
caa agc att gcc aac gca acc aat gag ctt gac cat cgt atc cac caa Gln Ser Ile Ala Asn Ala Thr Asn Glu Leu Asp His Arg Ile His Gln 1955 1960 1965	6445
aac gaa aat aag gcc aat gca ggg att tca tca gcg atg gcg atg gcg Asn Glu Asn Lys Ala Asn Ala Gly Ile Ser Ser Ala Met Ala Met Ala 1970 1975 1980	6493
tcc atg cca caa gcc tac att cct ggc aga tcc atg gtt acc ggg ggt Ser Met Pro Gln Ala Tyr Ile Pro Gly Arg Ser Met Val Thr Gly Gly 1985 1990 1995 2000	6541
att gcc acc cac aac ggt caa ggt gcg gtg gca gtg gga ctg tcg aag Ile Ala Thr His Asn Gly Gln Gly Ala Val Ala Val Gly Leu Ser Lys 2005 2010 2015	6589
ctg tcg gat aat ggt caa tgg gta ttt aaa atc aat ggt tca gcc gat Leu Ser Asp Asn Gly Gln Trp Val Phe Lys Ile Asn Gly Ser Ala Asp 2020 2025 2030	6637
acc caa ggc cat gta ggg gcg gca gtt ggt gca ggt ttt cac ttt Thr Gln Gly His Val Gly Ala Ala Val Gly Ala Gly Phe His Phe	6682

Ç

2035 2040 2045

taagccataa atcgcaagat tttacttaaa aatcaatctc accatagttg tataaaacag 6742 catcagcatc agtcatatta ctgatgctga tgtttttat cacttaaacc attttaccgc 6802 tcaagtgatt ctctttcacc atgaccaaat cgccattgat cataggtaaa cttattgagt 6862 aaattttatc aatgtagttg ttagatatgg ttaaaaattgt gccattgacc aaaaaatgac 6922 cgatttatcc cgaaaatttc tgattatgat ccgttgacct gcaggtcgac 6972

Figure 4. M. catarrhalis strain Q8 200kDa gene

					aaa Lys										48
	-			-	tat Tyr	_				_	_		 	_	96
					gtt Val										144
	_			_	gtc Val						_			_	192
_		_			att Ile 70										240
_				_	ctg Leu			_	_				 _	_	288
		_			agt Ser			_	_				_		336
					aaa Lys										384
					aaa Lys										432
_	_	_			gat Asp 150	_									480
	-		-		ctt Leu	_	_	_							528
					tta Leu										576
			_	_	aca Thr	_	_				_	_	_		624
	_	_			gca Ala	_						_			672

			gaa Glu									720
	_		caa Gln 245			_	_					768
			gcg Ala									816
			gcc Ala									864
			gcc Ala									912
			gcc Ala									960
		_	aat Asn 325									1008
			ggt Gly									1056
			gag Glu									1104
			aat Asn	Ser				Arg		Leu		1152
			ggt Gly									1200
		 _	aca Thr 405	_			_		_			1248
			ttg Leu									1296
_			aac Asn		_				_		 _	1344
			aca Thr									1392

-		_	_		act Thr 470		_	_		_		_			1440
					aaa Lys										1488
	_	_	_	_	aaa Lys				_		_	_		_	1536
		_			aac Asn	_		_	_		_				1584
					caa Gln	_		_					_		1632
_	_			_	gca Ala 550				_		_		_	_	1680
		_			att Ile			_							1728
					ttg Leu										1776
					agt Ser										1824
					ata Ile		Asn								1872
					cct Pro 630										1920
					gat Asp										1968
					agc Ser					_					2016
					aaa Lys										2064
_		-		_	aaa Lys	_	_	-	_						2112

690 695 700

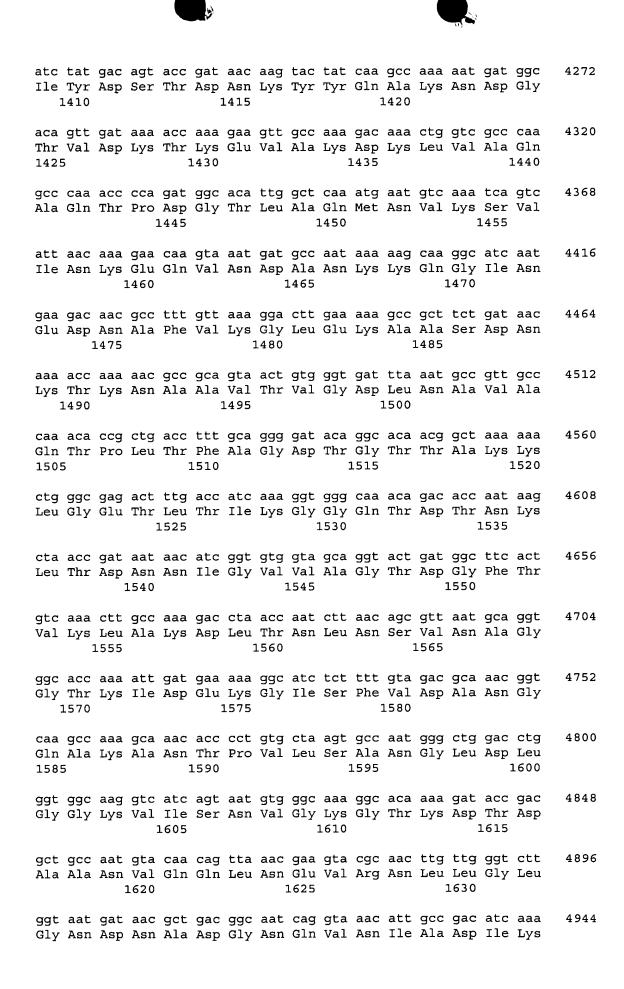
					gct Ala 710											2160
	_			_	atc Ile					_				_		2208
					ctc Leu			_			_			_		2256
	_	_			aca Thr						_		_			2304
					ctg Leu											2352
					gtc Val 790											2400
				_	tcc Ser											2448
	_			_	ctg Leu					_	_		_			2496
	_	_	_		gat Asp	_				_						2544
				-	aaa Lys	_		_				-				2592
					gcc Ala 870											2640
					gtg Val											2688
					gat Asp											2736
					aca Thr											2784
acc	gac	gat	gac	cat	gcc	ctt	gtt	aaa	gcc	agt	gat	atc	gcc	ggc	aat	2832





Thr Asp Asp Asp 930	His Ala Leu Val 935	Lys Ala Ser Asp 940	Ile Ala Gly Asn	
		cac acc acc aaa His Thr Thr Lys 955)
_	_	aaa aag gta gat Lys Lys Val Asp 970		3
		gtg ggt aaa gat Val Gly Lys Asp 985		5
		ggt aaa aac ggt Gly Lys Asn Gly		F
		ttt ggc att aac Phe Gly Ile Asn 1020		2
		cta aac aac aat Leu Asn Asn Asn 1035)
Lys Asn Thr Ala	_	atc caa gtc ggt Ile Gln Val Gly 1050		3
	Val Asn Asn Gly	gtt gta ggt gct Val Val Gly Ala 1065		5
		att ggc ttt act Ile Gly Phe Thr		Ė
		cta agc aaa gac Leu Ser Lys Asp 1100		<u> </u>
		caa tca ggt gag Gln Ser Gly Glu 1115)
Ser His Asp Ala		aag att tat gat Lys Ile Tyr Asp 1130		}
	Ile Ser Ser Thr	gcc aaa aca gca Ala Lys Thr Ala 1145		;
_		caa ggt aat aac Gln Gly Asn Asn	-	Ŀ

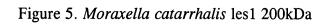
aac cct tac tcc agt tat gac acc tca aag acc tct gat gtc atc acc Asn Pro Tyr Ser Ser Tyr Asp Thr Ser Lys Thr Ser Asp Val Ile Thr 1170 1175 1180	3552
ttt gca ggt gaa aac ggc att acc acc aag gta aat aaa ggt gtg gtg Phe Ala Gly Glu Asn Gly Ile Thr Thr Lys Val Asn Lys Gly Val Val 1185 1190 1195 1200	3600
cgt gtg ggc att gac caa acc aaa ggc tta acc acg cct aag ctg acc Arg Val Gly Ile Asp Gln Thr Lys Gly Leu Thr Thr Pro Lys Leu Thr 1205 1210 1215	3648
gtg ggt aat aat aat ggc aaa ggc att gtc att aac agc caa aat ggt Val Gly Asn Asn Asn Gly Lys Gly Ile Val Ile Asn Ser Gln Asn Gly 1220 1225 1230	3696
caa aat acc atc aca gga cta agc aac act cta gct aat gtt acc aat Gln Asn Thr Ile Thr Gly Leu Ser Asn Thr Leu Ala Asn Val Thr Asn 1235 1240 1245	3744
gat aaa ggt agc gta cgc acc aca gaa cag ggc aat ata atc aaa gac Asp Lys Gly Ser Val Arg Thr Thr Glu Gln Gly Asn Ile Ile Lys Asp 1250 1255 1260	3792
gaa gac aaa acc cgt gcc gcc agc att gtt gat gtg cta agc gca ggc Glu Asp Lys Thr Arg Ala Ala Ser Ile Val Asp Val Leu Ser Ala Gly 1265 1270 1275 1280	3840
ttt aac ttg caa ggc aat ggt gaa gcg gtt gac ttt gtc tcc act tat Phe Asn Leu Gln Gly Asn Gly Glu Ala Val Asp Phe Val Ser Thr Tyr 1285 1290 1295	3888
gac acc gtc aac ttt gcc aat ggc aat acc acc acc gct aag gtg acc Asp Thr Val Asn Phe Ala Asn Gly Asn Thr Thr Thr Ala Lys Val Thr 1300 1305 1310	3936
tat gat gac aca agc aaa acc agt aaa gtg gtc tat gat gtc aat gtg Tyr Asp Asp Thr Ser Lys Thr Ser Lys Val Val Tyr Asp Val Asn Val 1315 1320 1325	3984
gat gat aca acc att gaa gtt aaa gat aaa aaa ctt ggc gta aaa acc Asp Asp Thr Thr Ile Glu Val Lys Asp Lys Lys Leu Gly Val Lys Thr 1330 1335 1340	4032
acc aca ttg acc agt act ggc aca ggt gct aat aaa ttt gcc cta agc Thr Thr Leu Thr Ser Thr Gly Thr Gly Ala Asn Lys Phe Ala Leu Ser 1345 1350 1355 1360	4080
aat caa gct act ggc gat gcg ctt gtc aag gcc agt gat atc gtt gct Asn Gln Ala Thr Gly Asp Ala Leu Val Lys Ala Ser Asp Ile Val Ala 1365 1370 1375	4128
cat cta aac acc tta tct ggc gac atc caa act gcc aaa ggg gca agc His Leu Asn Thr Leu Ser Gly Asp Ile Gln Thr Ala Lys Gly Ala Ser 1380 1385 1390	4176
caa gcg aac aac tca gca ggc tat gtg gat gct gat ggc aat aag gtc Gln Ala Asn Asn Ser Ala Gly Tyr Val Asp Ala Asp Gly Asn Lys Val 1395 1400 1405	4224



1635 1640 1645

aaa gac cca aat tca ggt tca tca tct aac cgc act gtc atc aaa gca Lys Asp Pro Asn Ser Gly Ser Ser Ser Asn Arg Thr Val Ile Lys Ala 1650 1655 1660	4992
ggc acg gta ctt ggc ggt aaa ggt aat aac gat acc gaa aaa ctt gcc Gly Thr Val Leu Gly Gly Lys Gly Asn Asn Asp Thr Glu Lys Leu Ala 1665 1670 1675 1680	5040
act ggt ggt gta caa gtg ggc gtg gat aaa gac ggc aac gct aac ggc Thr Gly Gly Val Gln Val Gly Val Asp Lys Asp Gly Asn Ala Asn Gly 1685 1690 1695	5088
gat tta agc aat gtt tgg gtc aaa acc caa aaa gat ggc agc aaa aaa Asp Leu Ser Asn Val Trp Val Lys Thr Gln Lys Asp Gly Ser Lys Lys 1700 1705 1710	5136
gcc ctg ctc gcc act tat aac gcc gca ggt cag acc aac tat gtg acc Ala Leu Leu Ala Thr Tyr Asn Ala Ala Gly Gln Thr Asn Tyr Val Thr 1715 1720 1725	5184
aac aac ccc gca gaa gcc att gac aga ata aat gaa caa ggt atc cgc Asn Asn Pro Ala Glu Ala Ile Asp Arg Ile Asn Glu Gln Gly Ile Arg 1730 1735 1740	5232
ttc ttc cat gtc aac gat ggc aat caa gag cct gtg gta caa ggg cgt Phe Phe His Val Asn Asp Gly Asn Gln Glu Pro Val Val Gln Gly Arg 1745 1750 1755 1760	5280
aac ggc att gac tca agt gcc tca ggc aag cac tca gtg gcg ata ggt Asn Gly Ile Asp Ser Ser Ala Ser Gly Lys His Ser Val Ala Ile Gly 1765 1770 1775	5328
ttc cag gcc aag gca gat ggt gaa gcc gcc gtt gcc ata ggc aga caa Phe Gln Ala Lys Ala Asp Gly Glu Ala Ala Val Ala Ile Gly Arg Gln 1780 1785 1790	5376
acc caa gca ggc aac caa tcc atc gcc atc ggt gat aac gca caa gcc Thr Gln Ala Gly Asn Gln Ser Ile Ala Ile Gly Asp Asn Ala Gln Ala 1795 1800 1805	5424
acg ggc gat caa tcc atc gcc atc ggt aca ggc aat gtg gta gca ggt Thr Gly Asp Gln Ser Ile Ala Ile Gly Thr Gly Asn Val Val Ala Gly 1810 1815 1820	5472
aag cac tot ggt gcc atc ggc gac cca agc act gtt aag gct gat aac Lys His Ser Gly Ala Ile Gly Asp Pro Ser Thr Val Lys Ala Asp Asn 1825 1830 1835 1840	5520
agt tac agt gtg ggt aat aac aac cag ttt acc gat gcc act caa acc Ser Tyr Ser Val Gly Asn Asn Asn Gln Phe Thr Asp Ala Thr Gln Thr 1845 1850 1855	5568
gat gtc ttt ggt gtg ggc aat aac atc acc gtg acc gaa agt aac tcg Asp Val Phe Gly Val Gly Asn Asn Ile Thr Val Thr Glu Ser Asn Ser 1860 1865 1870	5616
gtt gcc tta ggt tca aac tct gcc atc agt gca ggc aca cac gca ggc	5664

Val Ala Leu 1875	Gly Ser Asn	Ser Ala Ile 1880	_	Thr His Ala Gly 885	
_	Lys Lys Ser			acc acc aca gca Thr Thr Thr Ala	5712
				acg gcg gtt ggt Thr Ala Val Gly 1920	5760
		Ser Gly Ala		atc caa aat gtg Ile Gln Asn Val 1935	5808
Ala Ala Gly		_		gtc aat ggt agc Val Asn Gly Ser 1950	5856
	-	_	Ala Asn Ala	acc aat gag ctt Thr Asn Glu Leu 965	5904
	Ile His Gln			gca ggg att tca Ala Gly Ile Ser	5952
				att cct ggc aga Ile Pro Gly Arg 2000	6000
		Ile Ala Thr		caa ggt gcg gtg Gln Gly Ala Val 2015	6048
Ala Val Gly		-		tgg gta ttt aaa Trp Val Phe Lys 2030	6096
			His Val Gly	gcg gca gtt ggt Ala Ala Val Gly 045	6144
gca ggt ttt Ala Gly Phe 2050					6159



					aaa Lys:	_					_					48
					tgc Cys											96
					gtg Val											144
					atc Ile											192
_		_			aat Asn 70	_	_		_							240
	_		-	_	gct Ala	_		_	-			_			_	288
	_	_	_		gcc Ala			_		_				_		336
	_		_		caa Gln		-		_	_	-				_	384
					atc Ile											432
_	_	_		_	att Ile 150		-	_	_			_	_			480
_					tat Tyr						_					528
			_		cgc Arg			_	_					_		576
					gca Ala											624
				_	ggt Gly			_		_						672

	210				215				220				
			aac Asn										720
_			aca Thr 245		-				_		_		768
			gcc Ala										816
		_	ggt Gly				_						864
			gcc Ala										912
			acc Thr										960
	_	_	atc Ile 325			_		_					1008
			gca Ala										1056
			aag Lys		_	_				_	_		1104
			tta Leu										1152
			aat Asn										1200
_		_	ctt Leu 405	_				_		_		 _	1248
			acc Thr										1296
			cta Leu										1344

					aaa Lys									1392
	_				acg Thr 470	_	_	_		_				1440
_					agc Ser		_							1488
			_		gac Asp	_		_		_				1536
			_		gtt Val					 _				1584
					gat Asp									1632
_			_	_	aaa Lys 550				-		_		_	1680
					gct Ala									1728
					ctt Leu								gac` Asp	1776
_				_	ggt Gly	_				_				1824
-	-	-		_	gca Ala	_			_			_		1872
					gcc Ala 630									1920
	_	_		_	att Ile	_	_							1968
			-		atc Ile									2016

_		_		_				_		_	ggt Gly		_			2064
				_		_	_				agc Ser 700	_		_		2112
											ggt Gly					2160
											agc Ser					2208
											atc Ile					2256
_				_	_	_			_		tta Leu					2304
					_			_			gtc Val 780					2352
											gct Ala					2400
											gat Asp					2448
_				-			-				aag Lys					2496
	_					_					gct Ala					2544
			_			_		_	_		gtt Val 860		_		_	2592
	_	-						-	_	-	att Ile					2640
		_	_		_						gtc Val			_		2688
gca	act	gat	gac	gaa	acc	atc	acc	gtg	ggt	aaa	gat	ggt	aca	caa	aac	2736

Ala Thr Asp Asp		e Thr Val 905		Asp Gly Thr 910	Gln Asn
ggc aag acc gto Gly Lys Thr Va 915					
gtt gct acc aar Val Ala Thr Ass 930		y Thr Val	Thr Phe G		
agc ggt ctt aaa Ser Gly Leu Ly: 945					
tct att aaa aad Ser Ile Lys Asi					
ggc gtg aag tt Gly Val Lys Pho 980	Ala Lys Va				
gat ggc aca ago Asp Gly Thr Se: 995	-	_	_	_	
aat ggc tca ct Asn Gly Ser Let 1010	_	r Lys Pro	His Leu T	_	•
aaa gtg ggt gaa Lys Val Gly Glu 1025				_	
aaa aag att acc Lys Lys Ile Th		n Ser Gly	-	hr Gln Asn	_
gat gct gtg aca Asp Ala Val The 1060	Gly Gly A				
agc aaa atc aad Ser Lys Ile Asi 1075					
ttc tca gta gca Phe Ser Val Ala 1090		n Gly Asn	His Phe T		
tac tcc agt tag Tyr Ser Ser Tyr 1105					
ggt gaa aac ggo Gly Glu Asn Gly					

1125 1130 1135

ggc att gac caa Gly Ile Asp Gln 1140	Thr Lys Gly Leu			56
aat aat aat ggc Asn Asn Asn Gly 1155		Ile Asp Ser Lys		04
acc atc aca gga Thr Ile Thr Gly 1170	_	_	5 55	52
gca gga cac gca Ala Gly His Ala 1185				00
cgt gcc gcc agc Arg Ala Ala Ser 1				48
ggc aat ggt gaa Gly Asn Gly Glu 1220	Ala Val Asp Phe			96
ttt atc gat ggc Phe Ile Asp Gly 1235		Ala Lys Val Thr		44
agc aaa acc agt Ser Lys Thr Ser 1250			=	92
att gaa gtg aca Ile Glu Val Thr 1265				40
acc aaa aca agt Thr Lys Thr Ser 1		-		88
ggc gat gcc ctt Gly Asp Ala Leu 1300	Val Lys Ala Ser			36
ttg gct ggc gac Leu Ala Gly Asp 1315	_	Lys Gly Ala Ser	3 3 3	84
tca gca agc tat Ser Ala Ser Tyr 1330				32
acc gat aag aag Thr Asp Lys Lys 1345				80

aac aaa gaa gtt gcc aaa gac aaa ctg gtc gcc caa gcc caa acc cca Asn Lys Glu Val Ala Lys Asp Lys Leu Val Ala Gln Ala Gln Thr Pro 1365 1370 1375	4128
gat ggc aca ttg gct caa atg aat gtc aaa tca gtc att aac aaa gag Asp Gly Thr Leu Ala Gln Met Asn Val Lys Ser Val Ile Asn Lys Glu 1380 1385 1390	4176
caa gta aat gat gcc aat aaa aag caa ggc atc aat gaa gac aac gcc Gln Val Asn Asp Ala Asn Lys Lys Gln Gly Ile Asn Glu Asp Asn Ala 1395 1400 1405	4224
ttt atc aaa ggg ctt gaa aac gcc gcc aaa gac acc aaa acc aaa aac Phe Ile Lys Gly Leu Glu Asn Ala Ala Lys Asp Thr Lys Thr Lys Asn 1410 1415 1420	4272
gcc gca gta act gtg ggt gat tta aat gcc gtt gcc caa aca ccg ctg Ala Ala Val Thr Val Gly Asp Leu Asn Ala Val Ala Gln Thr Pro Leu 1425 1430 1435 1440	4320
acc ttt gca ggg gat aca ggc aca acg gct aaa aaa ctg ggc gag act Thr Phe Ala Gly Asp Thr Gly Thr Thr Ala Lys Lys Leu Gly Glu Thr 1445 1450 1455	4368
ttg acc atc aaa ggt ggg caa aca gac acc aat aag cta acc gat aat Leu Thr Ile Lys Gly Gly Gln Thr Asp Thr Asn Lys Leu Thr Asp Asn 1460 1465 1470	4416
aac atc ggt gtg gta gca ggt act gat ggc ttc act gtc aaa ctt gcc Asn Ile Gly Val Val Ala Gly Thr Asp Gly Phe Thr Val Lys Leu Ala 1475 1480 1485	4464
aaa gac cta acc aat ctt aac agc gtt aat gca ggt ggc acc aga att Lys Asp Leu Thr Asn Leu Asn Ser Val Asn Ala Gly Gly Thr Arg Ile 1490 1495 1500	4512
gat gaa aaa ggc atc tct ttt gta gac gca aac ggt caa gcc aaa gca Asp Glu Lys Gly Ile Ser Phe Val Asp Ala Asn Gly Gln Ala Lys Ala 1505 1510 1515 1520	4560
aac acc cct gtg cta agt gcc aat ggg ctg gac ctg ggt ggc aaa cgc Asn Thr Pro Val Leu Ser Ala Asn Gly Leu Asp Leu Gly Gly Lys Arg 1525 1530 1535	4608
atc agt aac atc ggt gca gct gtt gat gat aac gat gcg gtg aac ttt Ile Ser Asn Ile Gly Ala Ala Val Asp Asp Asn Asp Ala Val Asn Phe 1540 1545 1550	4656
aag cag ttt aat gaa gtt gcc aaa acg gtc aac aac cta aac aac caa Lys Gln Phe Asn Glu Val Ala Lys Thr Val Asn Asn Leu Asn Asn Gln 1555 1560 1565	4704
agt aac tca ggt gcg tca tta ccc ttt gtg gta acc gat gcc aat ggc Ser Asn Ser Gly Ala Ser Leu Pro Phe Val Val Thr Asp Ala Asn Gly 1570 1575 1580	4752

aag ccc atc aat ggc acc gat ggc aag ccc caa aaa gcc atc aag ggc Lys Pro Ile Asn Gly Thr Asp Gly Lys Pro Gln Lys Ala Ile Lys Gly 1585 1590 1595 1600	4800
gcc gat ggt aaa tac tat cac gcc aac gcc aac ggc gta cct gtg gac Ala Asp Gly Lys Tyr Tyr His Ala Asn Ala Asn Gly Val Pro Val Asp 1605 1610 1615	4848
aaa gat ggc aag ccc atc acc gat gcg gac aaa ctt gcc aat ctg gca Lys Asp Gly Lys Pro Ile Thr Asp Ala Asp Lys Leu Ala Asn Leu Ala 1620 1625 1630	4896
gct cat ggc aaa ccc ctt gat gca ggt cat caa gtg gtg gca agc cta Ala His Gly Lys Pro Leu Asp Ala Gly His Gln Val Val Ala Ser Leu 1635 1640 1645	4944
ggc ggc aac tca gat gcc atc acc cta acc aac atc aag tcc act ttg Gly Gly Asn Ser Asp Ala Ile Thr Leu Thr Asn Ile Lys Ser Thr Leu 1650 1655 1660	4992
cca caa att gac aca cca aac aca ggt aat gcc aat gca ggg caa gcc Pro Gln Ile Asp Thr Pro Asn Thr Gly Asn Ala Asn Ala Gly Gln Ala 1665 1670 1675 1680	5040
caa agt ctg ccc agc cta tca gca gca cag caa agt aat gct gcc agt Gln Ser Leu Pro Ser Leu Ser Ala Ala Gln Gln Ser Asn Ala Ala Ser 1685 1690 1695	5088
gtc aaa gat gtg cta aat gta ggc ttt aac ttg cag acc aat cac aat Val Lys Asp Val Leu Asn Val Gly Phe Asn Leu Gln Thr Asn His Asn 1700 1705 1710	5136
caa gtg gac ttt gtc aaa gcc tat gat acc gtc aac ttt gtc aat ggt Gln Val Asp Phe Val Lys Ala Tyr Asp Thr Val Asn Phe Val Asn Gly 1715 1720 1725	5184
aca ggt gcc gac atc aca agc gtg cgt agt gct gat ggc acg atg agt Thr Gly Ala Asp Ile Thr Ser Val Arg Ser Ala Asp Gly Thr Met Ser 1730 1735 1740	5232
aac atc acc gtc aac acc gcc tta gca gcg acc gat gat gat ggc aat Asn Ile Thr Val Asn Thr Ala Leu Ala Ala Thr Asp Asp Asp Gly Asn 1745 1750 1755 1760	5280
gtg ctt atc aaa gcc aaa gat ggt aag ttc tac aaa gca gac gac ctc Val Leu Ile Lys Ala Lys Asp Gly Lys Phe Tyr Lys Ala Asp Asp Leu 1765 1770 1775	5328
atg cca aac ggc tca cta aaa gca ggc aaa tca gcc agt gat gcc aaa Met Pro Asn Gly Ser Leu Lys Ala Gly Lys Ser Ala Ser Asp Ala Lys 1780 1785 1790	5376
act cca act ggt cta agc ctt gtt aac ccc aat gct ggt aaa ggc agt Thr Pro Thr Gly Leu Ser Leu Val Asn Pro Asn Ala Gly Lys Gly Ser 1795 1800 1805	5424
aca ggc gat gca gtg gct ctt aat aac tta tca aaa gcg gta ttt aaa	5472

Thr Gly Asp Ala Val Ala Leu Asn Asn Leu Ser Lys Ala Val Phe Lys 1810 1815 1820	
tcc aaa gat ggt aca act act acc aca gta agc tct gat ggc atc agt Ser Lys Asp Gly Thr Thr Thr Thr Thr Val Ser Ser Asp Gly Ile Ser 1825 1830 1835 1840	5520
atc caa ggc aaa gat aac agc agc atc acc cta agc aaa gat ggg ctg Ile Gln Gly Lys Asp Asn Ser Ser Ile Thr Leu Ser Lys Asp Gly Leu 1845 1850 1855	5568
aat gta ggc ggt aag gtc atc agc aat gtg ggt aaa ggc aca aaa gac Asn Val Gly Gly Lys Val Ile Ser Asn Val Gly Lys Gly Thr Lys Asp 1860 1865 1870	5616
acc gac gct gcc aat gta caa cag tta aac gaa gta cgc aac ttg ttg Thr Asp Ala Ala Asn Val Gln Gln Leu Asn Glu Val Arg Asn Leu Leu 1875 1880 1885	5664
ggt ctt ggt aat gct ggt aat gat aac gct gac ggc aat cag gta aac Gly Leu Gly Asn Ala Gly Asn Asp Asn Ala Asp Gly Asn Gln Val Asn 1890 1895 1900	5712
att gcc gac atc aaa aaa gac cca aat tca ggt tca tca tct aac cgc Ile Ala Asp Ile Lys Lys Asp Pro Asn Ser Gly Ser Ser Ser Asn Arg 1905 1910 1915 1920	5760
act gtc atc aaa gca ggc acg gta ctt ggc ggt aaa ggt aat aac gat Thr Val Ile Lys Ala Gly Thr Val Leu Gly Gly Lys Gly Asn Asn Asp 1925 1930 1935	5808
acc gaa aaa ctt gcc act ggt ggt gta caa gtg ggc gtg gat aaa gac Thr Glu Lys Leu Ala Thr Gly Gly Val Gln Val Gly Val Asp Lys Asp 1940 1945 1950	5856
ggc aac gct aac ggc gat tta agc aat gtt tgg gtc aaa acc caa aaa Gly Asn Ala Asn Gly Asp Leu Ser Asn Val Trp Val Lys Thr Gln Lys 1955 1960 1965	5904
gat ggc agc aaa aaa gcc ctg ctc gcc act tat aac gcc gca ggt cag Asp Gly Ser Lys Lys Ala Leu Leu Ala Thr Tyr Asn Ala Ala Gly Gln 1970 1975 1980	5952
acc aac tat ttg acc aac aac ccc gca gaa gcc att gac aga ata aat Thr Asn Tyr Leu Thr Asn Asn Pro Ala Glu Ala Ile Asp Arg Ile Asn 1985 1990 1995 2000	6000
gaa caa ggt atc cgc ttc ttc cat gtc aac gat ggc aat caa gag cct Glu Gln Gly Ile Arg Phe Phe His Val Asn Asp Gly Asn Gln Glu Pro 2005 2010 2015	6048
gtg gta caa ggg cgt aac ggc att gac tca agt gcc tca ggc aag cac Val Val Gln Gly Arg Asn Gly Ile Asp Ser Ser Ala Ser Gly Lys His 2020 2025 2030	6096
tca gtg gcg ata ggt ttc cag gcc aag gca gat ggt gaa gcc gcc gtt Ser Val Ala Ile Gly Phe Gln Ala Lys Ala Asp Gly Glu Ala Ala Val	6144

2035 2040 2045

gcc ata ggc ag Ala Ile Gly Ar 2050				6192
gat aac gca ca Asp Asn Ala Gl 2065		Asp Gln Ser		6240
aat gtg gta ac Asn Val Val Th				6288
gtt aag gct ga Val Lys Ala As 210	p Asn Ser Tyr		Asn Asn Asn	6336
gat gcc act ca Asp Ala Thr Gl 2115	n Thr Asp Val			 6384
acc gaa agt aa Thr Glu Ser As 2130				6432
ggc aca cac go Gly Thr His Al 2145		Ala Lys Lys		6480
aca acc acc ac Thr Thr Thr Th				6528
caa acg gcg gt Gln Thr Ala Va 218	l Gly Ala Val		Ala Ser Gly	6576
cgt atc caa aa Arg Ile Gln As 2195	n Val Ala Ala	Gly Glu Val		6624
gcg gtc aat gg Ala Val Asn Gl 2210		Tyr Lys Ala		6672
gca acc aat ga Ala Thr Asn Gl 2225		Arg Ile His		6720
aat gca ggg at Asn Ala Gly Il				6768
tac att cct go Tyr Ile Pro Gl 226	y Arg Ser Met		Gly Ile Ala	6816

 0 0 0 00	ctg tcg aag ctg tcg gat aat ggt Leu Ser Lys Leu Ser Asp Asn Gly 2285	6864
	tca gcc gat acc caa ggc cat gta Ser Ala Asp Thr Gln Gly His Val 2300	6912
 ggt gca ggt ttt Gly Ala Gly Phe 2310		6942

DGESTALO.CZEZAS

Figure 6. Alignment of amino acid sequence of 200kDa proteins of M. catarrhalis strains

4223	4223	4223	4223	4223	4223	4223	4223
Q8	Q8	Q8	Q8	Q8	Q8	Q8	
LES-1	LES-1	LES-1	LES-1	LES-1	LES-1	LES-1	
10 20 30 40 50 60 70 80 90 100 MNHIYKVIFNKATGTFMAVAEYAKSHSTGGGGSCATGQVGSVCTLSFARIAALAVLVIGATLSGSAYAQKKOTKHIAIGEQNQPRRSGTAKADGDRAIAIG	110 120 130 140 150 160 170 180 190 200 ENANAÇGÇAIAIGSSNKTVNGSSLD-KIGTDATGÇESIAIGGDVKASGDASIAIGSDDLHLLDQHGNPKHPKGTLINDLINGHAVLKEIRSSKDNDVKYR SLSKSVKPDP.NG.NG-NV.SH.K.NL.EY.PKNLDLNEFHKHEIK.QT.T.GKI SL.K.HANG.KPDPRNQAANQ.A.SH.K.KL.EY.DRNST.S.Y.N.L.STQN.T.RQD.NGSQ	210 220 240 250 260 270 280 290 300 RTTASGHASTAVGAMSYAQGHFSNAFGTRATAKSAYSLAVGLAATAEGQSTIAIGSDATSSSLGAIALGAGTRAQLQGSIALGQGSVVTQSDNNSRPAYTR.Q	310 320 340 360 360 400 PNTQALDPKFQATNNTKAGPL-SIG-SNSIKRKIINVGAGVNKTDAVNVAQLEAVVKWAKERRITFQGD-DN-STDVKIGLDNTLTIKGGAETNALTDNN-IGVV .LGKT.ADQYKRQGDSTDIFNNNNSRSRDKL.EELN.KKGN.NS.ERGD.QEAEGNGSNIKSS-KGNG.FSSTYEDX.ENLQKG.GKK.GEQ.DKN	#10 #20 #30 #440 #50 #60 #70 #80 #90 500 KEADNSGLKVKLAKTLANLTEVNTTTLNATTTVKVGSSSSTTAELLSDSLTFTQPNTGSQSTSKTVYGVNGVKFTNNAETTAAIGTTRITRDKIGFARDG TDGNE.TGSSNKIT.SNTNNNNQ.GGS-ITKDSID.LDSNSI.TKKKGTN TDNN-TN.SG.ET.S.KN.T.SEK.TGNNQ.GGT.NADTD.LD.SN.ALEDKSNKA	510 520 530 DVDEKQAPYLDKKQLKVGSVAITIDNGIDA GVDESKPYLDNEKLKVGNSTLNNGGLTVNNTTGNKQIQVGANGIKFATVANNVANTSATVGTARITEEKIGFAGTNDGERERR.ET.SN. GTVDENKPYLDKDKLKVGNSTLNNGGLTVNNTIGGSNKQIQVGADGIKFADVNVNVSN-AAKFGTTRITEEEIGFADADGKKSQG.KK.SN.	540 550 560 570 580 600 GNKKISNLAKGSSANDAVTIEQLKAAKPTLNAGAGISVTPTEISVDAKSGNVTAPTYNIGVKTTELNSDGHTG.TN.IANTKDINSNNGDLVDSI.TSKN.	610 620 630 640 650 660 670 680 690 700 TSDKFSVKGSGTNNSLVTAEHLASYLNEVNRTADS-ALQSFTVKEEDDDDANAITVAKDTTKNAGAVSILKLKGKNGLTVATKKD-GTVTFGLSQDSGLTIG

DOUGLETO LIVEVO

08 LES-1	4223 Q8 LES-1	4223 Q8 LES-1	4223 Q8 LES-1	4223 Q8 LES-1	4223 Q8 LES-1	4223 Q8 LES-1
GNNSNAHDKDDKEPK.QNGNSNGGKTFNTEVNIT.NRATID.SNTP	710 720 730 740 750 760 770 780 790 800 KSTLNNDGLTVKDTNEQIQVGANGIKFTNVNGSNPGTGIANTARITRDKIGFAGSDGAVDTNKPYLDQDKLQVGNVKITNTGINAGGKAITGLSPTLPSI.L.VGSDTNNR-LV-IVP-SADG.ST.NIIK	810 820 830 840 850 860 870 880 890 900 ADQSS-RNIELGNTIQ-DKDKSNAASINDILNTGFNLKNNNNPIDFVSTYDIVDFANGNATTATVTHDTANKTSKVVYDVNVDDTTIHLTGTDDNKKLGVKT .SP.GAE-ED.VAGKDKTIDY.EQAEKEG.KQ TNAGGV.TT.QTS.EKGS.SVGNTIDK.Y.ET.QTEKEG.TNKI	910 920 930 940 950 960 970 980 1000 TKLNKTSANGNTATNFNVNSSDED-ALVNAKDIAENINTLAKEIHTTKGTADTALQTFTVKKVDENNNADDANAITVGQKNANNQVNTLTLKGENGLNIKT ITET	1010 1020 1030 1050 1050 1060 1070 1080 1090 1100 DKNGTVTFGINTTSGLKAGKST-LANDGGLSIKNPTGSEQIQVGADGVKFAKVNNNGVVGAGIDGTTRITRDEIGFTGTNGSLDKSKPHLSKDGINAGGKKID	1110 1120 1130 1140 1150 1160 1170 1190 1200 TNIQSGEIAQNSHDAVTGGKIYDLKTELENKISSTAKTAQNSLHEFSVADEQGNNFTVSNPYSSYDTSKTSDVITFAGENGITTKVNKGVVRVGIDQTKG K	1210 1220 1230 1240 1250 1260 1270 1280 1390 1300 LTTPKLIVGNNNGKGIVIDSQNGQNTITGLSNTLANVTNDKGSVRTTEQGNIIKDEDKTRAASIVDVLSAGFNLQGNGEAVDFVSTYDTVNFADGNATTA N. T
GNNSNAHDKDDK	720 KSTLINNDGLTVKDTNEQIQVC .L.VGSDTNNR-LV-IVP-SAL .L.VGSDKDK.QLV-IVASG	810 820 83C ADQSS-RNIELGNTIQ-DKDKSNAASINDILA.SP.GAE-ED.V TNAGGV.TT.QTS.EKG	910 920 930 TKLNKTSANGNTATNFNVNSSDED-ALVNAKI ITETTTK.S.	1010 1020 1030 DKNGTVTFGINTTSGLKAGKST-LNDGGLSINDQDTNN	1110 1120 1130 TNIQSGEIAQNSHDAVTGGKIYDLKTELENKI	1210 1220 1230 LTTPKLTVGNNNGKGIVIDSQNGQNTITGLSN N

DSSEETS DSEEDS

1400 NKVI	: :)0 T.				£	, , <u>6</u> 4	Q
14 VDADGNK		1500 AVAQTPLT		1590 ANGLDL		NLAAHGK	3ADITSV	SSALLL
1390 SQANNSAGYY		1490 AAVTVGDLN?		1590 PVLSANGLDI	, , , , , , , , , , , , , , , , , , ,	PITDADKLAN	DTVNFVNGTC	AVFKSKDGT
1380 SGDIQTAKGA	• • •	1480 KAASDNKTKN	N . K. T	1540 1550 1560 1570 1580 1590 VAGTDGFTVKLAKDLTNLNSVNAGGTKIDDKGVSFVDSSGQAKANTPVLSANGLDL		NGVPVDKDGK	ANAGQAQSLPSLSAAQQSNAASVKDVLNVGFNLQTNHNQVDFVKAYDTVNFVNGTGADITSVR	 DAVALINNLSK
1370 SDIVAHLNTL	K.SANG.ATKF.ADATA.	1470 EDNAFVKGLE	INK.T.	1560 1570 158 SVNAGGTKIDDKGVSFVDSSGQAKA EIAN	! 1 1 3 1 0 1 0 1 1 1 1 1 1	ADGKYYHANA	LNVGFNLQTW	
1360 QATGDALVKA	D	1460 NDANKKQGIN	· · · · · · · · · · · · · · · · · · ·	1560 NLNSVNAGGT	\$ 1	DGKPQKAI KG	OSNAASVKOV	
1350 TGANKFALSN	ANG.ATKF.A	1450 VKSVINKEQV		1550 FTVKLAKDLT		DANGKPINGT	JSLPSLSAAQ	SLKAGKSASD
1340 VKTTTLTSTG		1440 TPDGTLAQMN		1540 NIGVVAGTDG		SGASLPFVVT	 	YKADDLMPNG
1330 TEVK-DKKLG	TS	1430 AKDKLVAQAQ		1530 TIDTNKLTDN-	1 1 1 1 1 1 1 1	TVNNLANQSN	KSTLPQIDTP	······································
1320 VYDVNVDDTT	NKTS	1420 IDGTVDKTKEV	JK.QN	1520 GETLTIKGGQ		nfk <u>o</u> fnevak	""; "NSDAITLTNI	
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 KVTYDDTSKTSKVVYDVNVDDTTIEVK-DKKLGVKTTTLTSTGTGANKFALSNQATGDALVKASDIVAHLNTLSGDIQTAKGASQANNSAGYVDADGNKVI		1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 YDSTDNKYYQAKNDGTVDKTKEVAKDKLVAQAQTPDGTLAQMNVKSVINKEQVNDANKKQGINEDNAFVKGLEKAASDNKTKNAAVTVGDLNAVAQTPLT	KVNDK.QN.	1510 1520 1530 FAGDTGTTAKK-LGETLTIKGGQTDTNKLTDN-NIGV		SNI GAAVDDNDAVNFKQFNEVAKTYNNLANNQSNSGASLPFVVTDANGKPINGTDGKPQKAI KGADGKYYHANANGVPVDKDGKPI TDADKLANLAAHGKP	LDAĞHQVVASLGGNSDAİTLTNİKSTLPQIDTPNTGN	SADGTMSNITVNTALAATDDDGNVLIKAKDGKFYKADDLMPNGSLKAGKSASDAKTPTGLSLVNPNAGKGSTGDAVALNNLSKAVFKSKDGTTTTTVSSD

4223 Q8 LES-1 4223 Q8 LES-1 LES-1 4223 Q8 LES-1 4223 Q8 LES-1

DOIGIELS . DIEIS

4223	4223	4223	4223	4223	4223
Q8	Q8	Q8	Q8	Q8	Q8
LES-1	LES-1	LES-1	LES-1	LES-1	LES-1
1600 GGKVISNVG 	1610 1620 1630 1640 1650 1660 1670 1680 1700 KGTKDTDAANVQQLNEVRNLLGLGNAADDADGNQVNIADIKKDPNSGSSSNRTVIKAGTVLGGKGNNDTEKLATGGIQVGVDKDGNANGDLSNVWVKTQ	1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 , KDGSKKALLATYNAAGQTNYLTNNPAEAIDRINEQGIRFFHVNDGNQEPVVQGRNGIDSSASGKHSVAIGFQAKADGEAAVAIGRQTQAGNQSIAIGDNA	1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 QATGDQSIAIGTGNVVAGKHSGAIGDPSTVKADNSYSVGNNNOFTDATQTDVFGVGNNITVTESNSVALGSNSAISAGTHAGTQAKKSDGTAGTTTTAGA	1910 1920 1940 1950 1960 1970 1980 1990 2000 TGTVKGFAGQTAVGAVSVGASGAERRIQNVAAGEVSATSTDAVNGSQLYKATQSIANATNELDHRIHQNENKANAGISSAMAMASMPQAYIPGRSMVTGG	2010 2020 2040 IATHNGQGAVAVGLSKLSDNGQWVFKINGSADTQGHVGAAVGAGFHF* *********************************

Construction of Plasmids Expressing Portions of the 200 kDa Protein Gene from Strain 4223

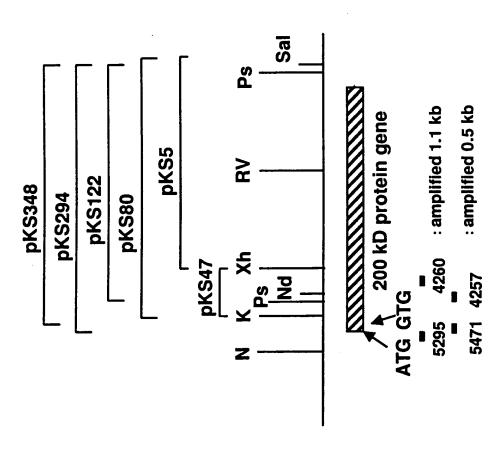


Figure 8. M. catarrhalis M56 200kDa gene in pKS348.

			_	_		_		_	-		_	caa Gln		_	48
				_			_			_		aga Arg			96
												ggt Gly 45			144
	_	_				_		_			_	agt Ser			192
_			_	_	_	_						gct Ala			240
												ggt Gly			288
	_			_	_	-			-		_	cag Gln			336
												att Ile 125			384
												gat Asp			432
												gtg Val			480
												aca Thr			528
												gcc Ala			576
												tct Ser 205			624
												cta Leu			672

210 215 220 att gcc cta ggt caa ggt tct gtt gtc act cag agt gat aat aat tct 720 Ile Ala Leu Gly Gln Gly Ser Val Val Thr Gln Ser Asp Asn Asn Ser 230 235 aga ccg gcc tat aca cca aat acc cag gca cta gac ccc aag ttt caa 768 Arg Pro Ala Tyr Thr Pro Asn Thr Gln Ala Leu Asp Pro Lys Phe Gln 245 250 gcc acc aat aat acg aag gcg ggt cca ctt tcc att ggt agt aac tct 816 Ala Thr Asn Asn Thr Lys Ala Gly Pro Leu Ser Ile Gly Ser Asn Ser 260 atc aaa cgt aaa atc atc aat gtc ggt gca ggt gtt aat aaa acc gat 864 Ile Lys Arg Lys Ile Ile Asn Val Gly Ala Gly Val Asn Lys Thr Asp 275 gcg gtc aat gtg gca cag cta gaa gcg gtg gtg aag tgg gct aag gag 912 Ala Val Asn Val Ala Gln Leu Glu Ala Val Val Lys Trp Ala Lys Glu 290 295 cgt aga att act ttt cag ggt gat gat aac agt act gac gta aaa ata 960 Arg Arg Ile Thr Phe Gln Gly Asp Asp Asn Ser Thr Asp Val Lys Ile 1008 ggt ttg gat aat act tta act att aaa ggt ggt gca gag acc aac gca Gly Leu Asp Asn Thr Leu Thr Ile Lys Gly Gly Ala Glu Thr Asn Ala 325 330 tta acc qat aat atc qqt qtq qta aaa qaq gct qat aat agt gqt 1056 Leu Thr Asp Asn Asn Ile Gly Val Val Lys Glu Ala Asp Asn Ser Gly 340 345 ctg aaa gtt aaa ctt gct aaa act tta aac aat ctt act gag gtg aat 1104 Leu Lys Val Lys Leu Ala Lys Thr Leu Asn Asn Leu Thr Glu Val Asn 355 360 aca act aca tta aat gcc aca acc aca gtt aag gta ggt agt agt agt 1152 Thr Thr Leu Asn Ala Thr Thr Val Lys Val Gly Ser Ser Ser 370 375 agt act aca gct gaa tta ttg agt gat agt tta acc ttt acc cag ccc 1200 Ser Thr Thr Ala Glu Leu Leu Ser Asp Ser Leu Thr Phe Thr Gln Pro 395 aat aca ggc agt caa agc aca agc aaa acc gtc tat ggc gtt aat ggg 1248 Asn Thr Gly Ser Gln Ser Thr Ser Lys Thr Val Tyr Gly Val Asn Gly gtg aag ttt act aat gca gaa aca gca gca atc ggc act act 1296 Val Lys Phe Thr Asn Asn Ala Glu Thr Thr Ala Ala Ile Gly Thr Thr 420 cqt att acc aga gat aaa att ggc ttt gct cga gat ggt gat gtt gat 1344 Arg Ile Thr Arg Asp Lys Ile Gly Phe Ala Arg Asp Gly Asp Val Asp 435 440

										ggt Gly		1392
_	_			_						aag Lys		1440
										atc Ile 495		1488
										atc Ile		1536
										gtt Val		1584
										agt Ser		1632
										aat Asn		1680
										aat Asn 575		1728
										gac Asp		1776
_	_	_	_			 _	_	_		aat Asn		1824
										acg Thr		1872
										gat Asp		1920
	_				_			_	 _	act Thr 655	_	1968
										aaa Lys		2016

					_							_		acc Thr	_	2064
														gca Ala		2112
_							_		_	_			_	ggc Gly		2160
														atc Ile 735		2208
	_				_		-		_	_		_	_	cgc Arg		2256
	_	_						_		_				gct Ala	_	2304
_			_											aat Asn		2352
			_		_				_		_	_		gcc Ala		2400
		_			_		-			_		_		aaa Lys 815		2448
		Val	Val	Tyr	Asp		Asn	Val	Asp	Āsp	Thr	Thr	Ile	cat His		2496
			_	_										aaa Lys		2544
			-	_					_					gtt Val		2592
														gaa Glu		2640
				_	_	_								gca Ala 895		2688
acc	gcc	cta	caa	acc	ttt	acc	gtt	aaa	aag	gta	gat	gaa	aat	aat	aat	2736

Thr Al	la Leu	Gln 900	Thr	Phe	Thr	Val	Lys 905	Lys	Val	Asp	Glu	Asn 910	Asn	Asn	
	at gac sp Asp 915														2784
Gln Va	tc aac al Asn 30														2832
	ac aaa sp Lys														2880
	aa gcc ys Ala			_				_			_				2928
	cc act ro Thr														2976
_	cc aag la Lys 995	_			Asn		_	-		Āla			_		3024
	ct cgc hr Arg 10			Arg					Phe						3072
	tt gat eu Asp		Ser					Ser					Asn		3120
	gt aaa ly Lys	Lys					Gln					Ala			3168
	at gat is Asp					Gly					Leu				3216
	aa aac lu Asn 1075				Ser					Ala					3264
	aa ttc lu Phe 90			Ala					Asn						3312
	ct tac ro Tyr		Ser					Lys					Ile		3360
_	ca ggt la Gly	_						_	_						3408

1125 1130 1135

cgt gtg ggc att Arg Val Gly Ile 1140	Asp Gln Thr			s Leu Thr
gtg ggt aat aat Val Gly Asn Asn 1155	Asn Gly Lys			
caa aat acc atc Gln Asn Thr Ile 1170				
gat aaa ggt agc Asp Lys Gly Ser 1185		Thr Glu Gln		
gaa gac aaa acc Glu Asp Lys Thr			Asp Val Leu Se	
ttt aac ttg caa Phe Asn Leu Gln 1220	Gly Asn Gly			r Thr Tyr
gac acc gtc aac Asp Thr Val Asn 1235	Phe Ala Asp			
tat gat gac aca Tyr Asp Asp Thr 1250				
gat gat aca acc Asp Asp Thr Thr 1265		Lys Asp Lys		
acc aca ttg acc Thr Thr Leu Thr	agt act ggc Ser Thr Gly 1285	Thr Gly Ala	Asn Lys Phe Al	c cta agc 3888 a Leu Ser 1295
aat caa gct act Asn Gln Ala Thr 1300	Gly Asp Ala			e Val Ala
cat cta aac acc His Leu Asn Thr 1315	Leu Ser Gly			
caa gcg aac aac Gln Ala Asn Asn 1330				
atc tat gac agt Ile Tyr Asp Ser 1345		Lys Tyr Tyr	caa gcc aaa aa Gln Ala Lys As 1355	

aca gtt gat aaa acc aaa gaa gtt gcc aaa gac aaa ctg gtc gcc caa Thr Val Asp Lys Thr Lys Glu Val Ala Lys Asp Lys Leu Val Ala Gln 1365 1370 1375	4128
gcc caa acc cca gat ggc aca ttg gct caa atg aat gtc aaa tca gtc Ala Gln Thr Pro Asp Gly Thr Leu Ala Gln Met Asn Val Lys Ser Val 1380 1385 1390	4176
att aac aaa gaa caa gta aat gat gcc aat aaa aag caa ggc atc aat Ile Asn Lys Glu Gln Val Asn Asp Ala Asn Lys Lys Gln Gly Ile Asn 1395 1400 1405	4224
gaa gac aac gcc ttt gtt aaa gga ctt gaa aaa gcc gct tct gat aac Glu Asp Asn Ala Phe Val Lys Gly Leu Glu Lys Ala Ala Ser Asp Asn 1410 1415 1420	4272
aaa acc aaa aac gcc gca gta act gtg ggt gat tta aat gcc gtt gcc Lys Thr Lys Asn Ala Ala Val Thr Val Gly Asp Leu Asn Ala Val Ala 1425 1430 1435 1440	4320
caa aca ccg ctg acc ttt gca ggg gat aca ggc aca acg gct aaa aaa Gln Thr Pro Leu Thr Phe Ala Gly Asp Thr Gly Thr Thr Ala Lys Lys 1445 1450 1455	4368
ctg ggc gag act ttg acc atc aaa ggt ggg caa aca gac acc aat aag Leu Gly Glu Thr Leu Thr Ile Lys Gly Gly Gln Thr Asp Thr Asn Lys 1460 1465 1470	4416
cta acc gat aat aac atc ggt gtg gta gca ggt act gat ggc ttc act Leu Thr Asp Asn Asn Ile Gly Val Val Ala Gly Thr Asp Gly Phe Thr 1475 1480 1485	4464
gtc aaa ctt gcc aaa gac cta acc aat ctt aac agc gtt aat gca ggt Val Lys Leu Ala Lys Asp Leu Thr Asn Leu Asn Ser Val Asn Ala Gly 1490 1495 1500	4512
ggc acc aaa att gat gac aaa ggc gtg tct ttt gta gac tca agc ggt Gly Thr Lys Ile Asp Asp Lys Gly Val Ser Phe Val Asp Ser Ser Gly 1505 1510 1515 1520	4560
caa gcc aaa gca aac acc cct gtg cta agt gcc aat ggg ctg gac ctg Gln Ala Lys Ala Asn Thr Pro Val Leu Ser Ala Asn Gly Leu Asp Leu 1525 1530 1535	4608
ggt ggc aag gtc atc agt aat gtg ggc aaa ggc aca aaa gat acc gac Gly Gly Lys Val Ile Ser Asn Val Gly Lys Gly Thr Lys Asp Thr Asp 1540 1545 1550	4656
gct gcc aat gta caa cag tta aac gaa gta cgc aac ttg ttg ggt ctt Ala Ala Asn Val Gln Gln Leu Asn Glu Val Arg Asn Leu Leu Gly Leu 1555 1560 1565	4704
ggt aat gct ggt aat gat aac gct gac ggc aat cag gta aac att gcc Gly Asn Ala Gly Asn Asp Asn Ala Asp Gly Asn Gln Val Asn Ile Ala 1570 1580	4752

gac atc aaa aaa gac cca aat tca ggt tca tca tct aac cgc act gtc Asp Ile Lys Lys Asp Pro Asn Ser Gly Ser Ser Ser Asn Arg Thr Val 1585 1590 1595 1600	4800
atc aaa gca ggc acg gta ctt ggc ggt aaa ggt aat aac gat acc gaa Ile Lys Ala Gly Thr Val Leu Gly Gly Lys Gly Asn Asn Asp Thr Glu 1605 1610 1615	4848
aaa ctt gcc act ggt ggt ata caa gtg ggc gtg gat aaa gac ggc aac Lys Leu Ala Thr Gly Gly Ile Gln Val Gly Val Asp Lys Asp Gly Asn 1620 1625 1630	4896
gct aac ggc gat tta agc aat gtt tgg gtc aaa acc caa aaa gat ggc Ala Asn Gly Asp Leu Ser Asn Val Trp Val Lys Thr Gln Lys Asp Gly 1635 1640 1645	4944
agc aaa aaa gcc ctg ctc gcc act tat aac gcc gca ggt cag acc aac Ser Lys Lys Ala Leu Leu Ala Thr Tyr Asn Ala Ala Gly Gln Thr Asn 1650 1655 1660	4992
tat ttg acc aac aac ccc gca gaa gcc att gac aga ata aat gaa caa Tyr Leu Thr Asn Asn Pro Ala Glu Ala Ile Asp Arg Ile Asn Glu Gln 1665 1670 1675 1680	5040
ggt atc cgc ttc ttc cat gtc aac gat ggc aat caa gag cct gtg gta Gly Ile Arg Phe Phe His Val Asn Asp Gly Asn Gln Glu Pro Val Val 1685 1690 1695	5088
caa ggg cgt aac ggc att gac tca agt gcc tca ggc aag cac tca gtg Gln Gly Arg Asn Gly Ile Asp Ser Ser Ala Ser Gly Lys His Ser Val 1700 1705 1710	5136
gcg ata ggt ttc cag gcc aag gca gat ggt gaa gcc gcc gtt gcc ata Ala Ile Gly Phe Gln Ala Lys Ala Asp Gly Glu Ala Ala Val Ala Ile 1715 1720 1725	5184
ggc aga caa acc caa gca ggc aac caa tcc atc gcc atc ggt gat aac Gly Arg Gln Thr Gln Ala Gly Asn Gln Ser Ile Ala Ile Gly Asp Asn 1730 1735 1740	5232
gca caa gcc acg ggc gat caa tcc atc gcc atc ggt aca ggc aat gtg Ala Gln Ala Thr Gly Asp Gln Ser Ile Ala Ile Gly Thr Gly Asn Val 1745 1750 1755 1760	5280
gta gca ggt aag cac tct ggt gcc atc ggc gac cca agc act gtt aag Val Ala Gly Lys His Ser Gly Ala Ile Gly Asp Pro Ser Thr Val Lys 1765 1770 1775	5328
gct gat aac agt tac agt gtg ggt aat aac aac cag ttt acc gat gcc Ala Asp Asn Ser Tyr Ser Val Gly Asn Asn Asn Gln Phe Thr Asp Ala 1780 1785 1790	5376
act caa acc gat gtc ttt ggt gtg ggc aat aac atc acc gtg acc gaa Thr Gln Thr Asp Val Phe Gly Val Gly Asn Asn Ile Thr Val Thr Glu 1795 1800 1805	5424
agt aac teg gtt gee tta ggt tea aac tet gee ate agt gea gge aca	5472

Ser Asn Ser Val Ala Leu Gly Ser Asn Ser Ala Ile Ser Ala Gly Thr 1810 1815 1820	
cac gca ggc aca caa gcc aaa aaa tct gac ggc aca gca ggt aca acc His Ala Gly Thr Gln Ala Lys Lys Ser Asp Gly Thr Ala Gly Thr Thr 1825 1830 1835 1840	520
acc aca gca ggt gca acc ggt acg gtt aaa ggc ttt gct gga caa acg Thr Thr Ala Gly Ala Thr Gly Thr Val Lys Gly Phe Ala Gly Gln Thr 1845 1850 1855	5568
gcg gtt ggt gcg gtc tcc gtg ggt gcc tca ggt gct gaa cgc cgt atc Ala Val Gly Ala Val Ser Val Gly Ala Ser Gly Ala Glu Arg Arg Ile 1860 1865 1870	616
caa aat gtg gca gca ggt gag gtc agt gcc acc agc acc gat gcg gtc Gln Asn Val Ala Ala Gly Glu Val Ser Ala Thr Ser Thr Asp Ala Val 1875 1880 1885	6664
aat ggt agc cag ttg tac aaa gcc acc caa agc att gcc aac gca acc Asn Gly Ser Gln Leu Tyr Lys Ala Thr Gln Ser Ile Ala Asn Ala Thr 1890 1895 1900	712
aat gag ctt gac cat cgt atc cac caa aac gaa aat aag gcc aat gca Asn Glu Leu Asp His Arg Ile His Gln Asn Glu Asn Lys Ala Asn Ala 1905 1910 1915 1920	760
ggg att tca tca gcg atg gcg atg gcg tcc atg cca caa gcc tac att Gly Ile Ser Ser Ala Met Ala Met Ala Ser Met Pro Gln Ala Tyr Ile 1925 1930 1935	808
cct ggc aga tcc atg gtt acc ggg ggt att gcc acc cac aac ggt caa 5 Pro Gly Arg Ser Met Val Thr Gly Gly Ile Ala Thr His Asn Gly Gln 1940 1945 1950	856
ggt gcg gtg gca gtg gga ctg tcg aag ctg tcg gat aat ggt caa tgg 59 Gly Ala Val Ala Val Gly Leu Ser Lys Leu Ser Asp Asn Gly Gln Trp 1955 1960 1965	904
gta ttt aaa atc aat ggt tca gcc gat acc caa ggc cat gta ggg gcg 59 Val Phe Lys Ile Asn Gly Ser Ala Asp Thr Gln Gly His Val Gly Ala 1970 1975 1980	952
gca gtt ggt gca ggt ttt cac ttt taagccataa atcgcaagat tttacttaaa 6 Ala Val Gly Ala Gly Phe His Phe 1985 1990	006
aatcaatctc accatagttg tataaaacag catcagcatc agtcatatta ctgatgctga 6	066
tgttttttat cacttaaacc attttaccgc tcaagtgatt ctctttcacc atgaccaaat 6	126
cgccattgat cataggtaaa cttattgagt aaattttatc aatgtagttg ttagatatgg 6	186
ttaaaattgt gccattgacc aaaaaatgac cgatttatcc cgaaaatttc tgattatgat 6	246
ccgttgacct gca 6	259

Figure 9A Construction of pKS294

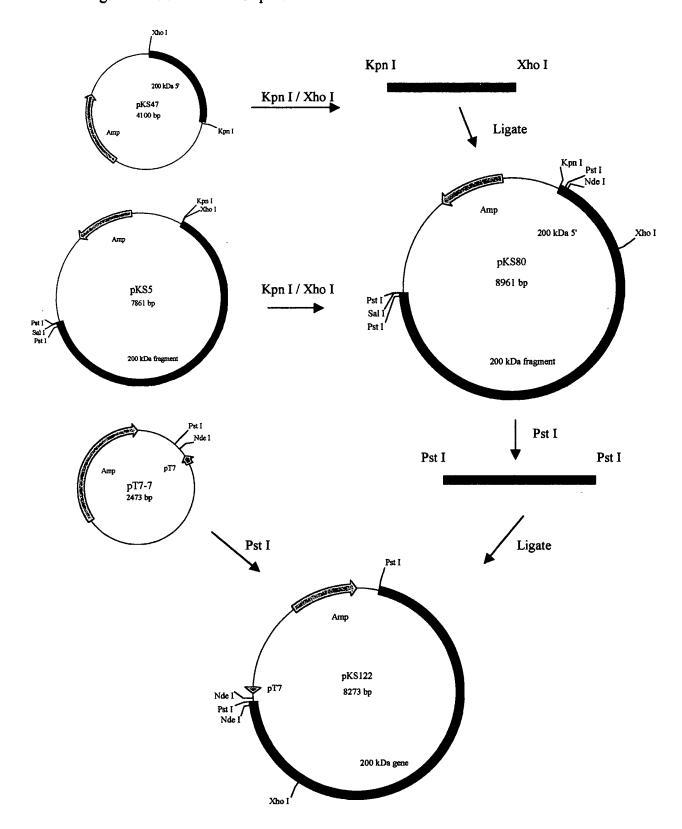


Figure 9B Construction of pKS294

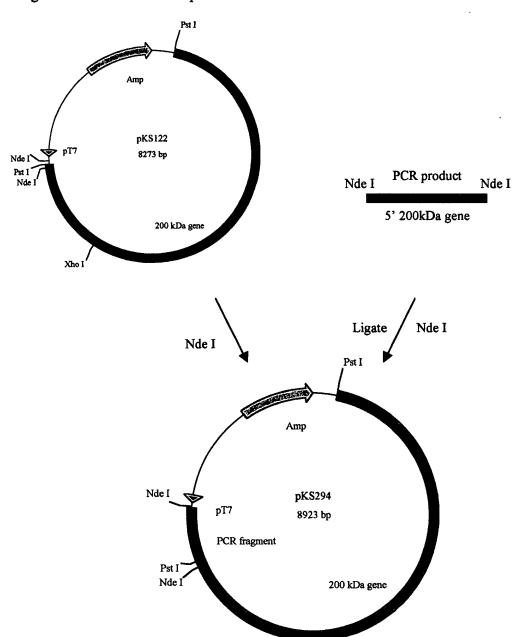


Figure 10. Construction of pKS348

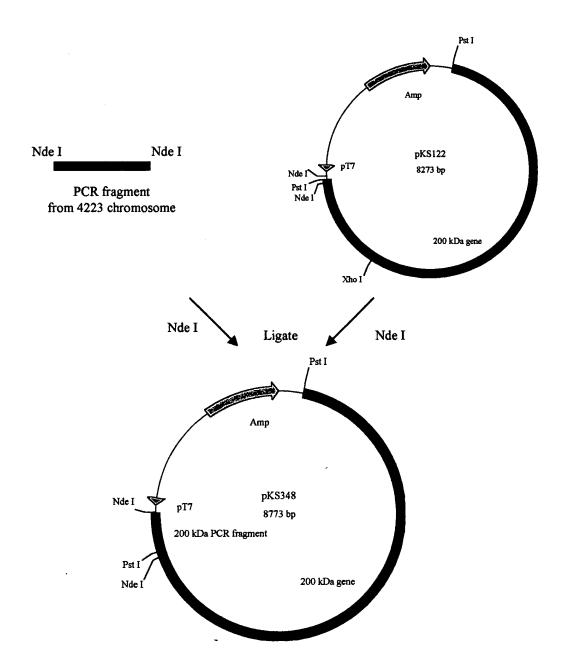


FIGURE 11

Purification of r200 kDa Protein from E. coli

E. coli Whole Cell

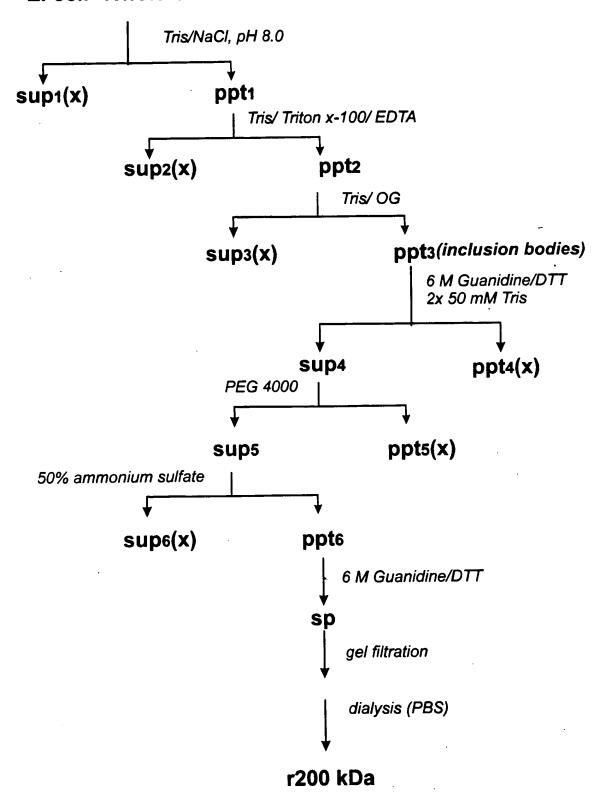
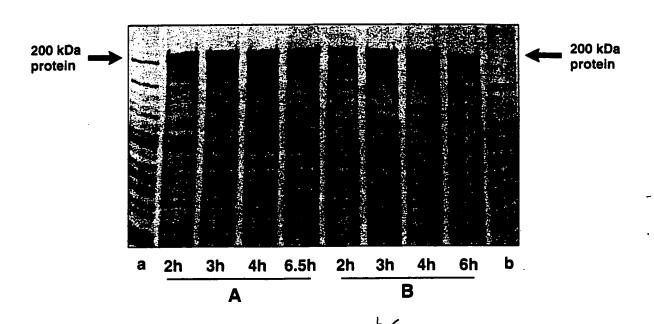


FIGURE 12

Expression of M56 r200 kDa Protein Gene in *E. coli*



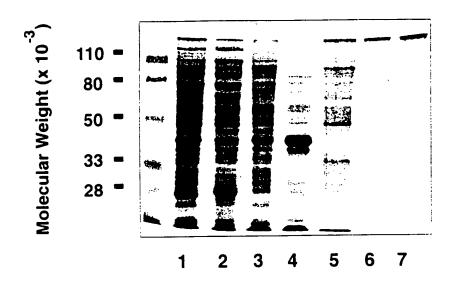
A: KS358 induced when O.D. 600 nm was 0.26

B: KS358 induced when O.D. at 600 nm was 0.44

a: strain 4223 lysate

b: KS358 cultured overnight

FIGURE 13
Purification of M56 r200 kDa Protein (4223)



- 1. E. coli Whole cells
- 2. Soluble proteins after 50 mM Tris/ NaCl, pH 8, extraction
- 3. Soluble proteins after Tris/ Triton X-100/EDTA extraction
- 4. Soluble proteins after Tris/ OG extraction
- 5. Pellet after Tris/ OG extraction
- 6-7. Purified 200 kDa protein

FIGURE 14

Anti-M56 r200 kDa Antibody Titers in Mice

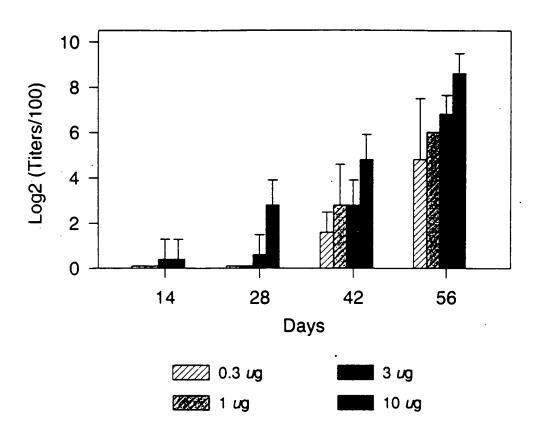


FIGURE 15
Anti-M56 r200 kDa Antibody Titers in Guinea Pigs

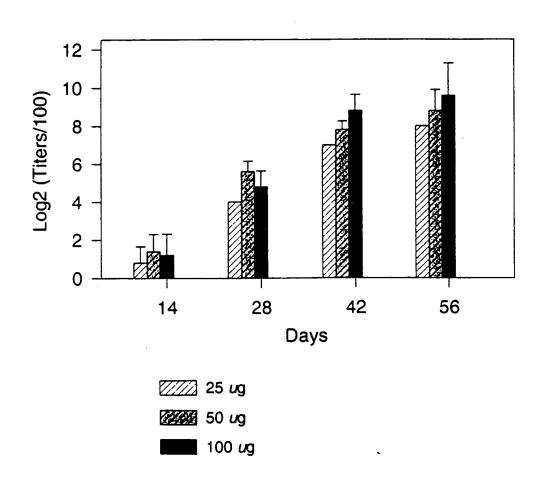


FIGURE 16

PCR amplification of DNA fragments carrying a portion of the 200 kDa protein gene from chromosomal DNA of RH408

	: amplified 2.3 k	: amplified 2.3 k	: amplified 2.0 k	: amplified 2.3 kt
le le			267	498 1
protein ger		4272		4269
200 kD	4278	4329	4	
ATG	4254			
	ATG 200 kD protein gene		200 kD protein gene	200 kD protein gene 4278 4329 4118 4267

Figure 17

M. catarrhalis strain 4223 200 kDa

IVI.	ca	ıaı	rrn	au	3 3	u	1111	۱ 🛨		. د.	20	UF	T.	a																				
CC	A T	G G	АТ		G	G G	C A	G	GТ			T	C G	C	СТ			3 Т	A :	r G	ΑI			g a	т (G A	C A			C A	. т т	'T G		•
				10						20						30						40)					50)				•	60
								_				_		_																				_
C A	TA	TC	ΤG	ТА 70	C	3 A	тт	T	G A	80		; T (G A	т.	ΑТ	90	Т:	ГТ	A A	A C	ΑТ	100		A C	A:	ľG	AI	110		A C	АТ	TG		Т 20
A A '	ΤA	ст	GТ	тG	c	2 A	T C	A '	тт	A C	C A	T	A' A	т '	тт	A G	T A	A A	c c	3 C	ΑТ	тт	A	3 T	A A	, c	G C	AI	т'	T G	T A	AA	A A	T
				130						140						150						160						170	;				18	30
CA	ТТ	G C	GC	C C	C 1	T	T A	T (G T	G T 200		C Z	A T	A '	T G	A A 210	T F	A G	A A	AT.	ΑТ	T A 220	T	3 A	TI	G	TA	T C		3 A	тт	ΑT	T G 24	
				20						200						210												250						
АТ	C A	G A	АТ	GG	то	A	T G	c :	ГА	ΤA	тс	A	ГG	A '	r G	c c	T F	A C	G A	. G	тт	GA	TI	ГŤ	GG	G	тт	A A	T	C A	СТ	СТ	АТ	G
				250						260						270						280						290						00_
A T	гт	g a	T A		T I	T	T G	A Z	A A			т (C T	A '	ГТ		C 1	rT	A A	A	тС			A T	A 1	G	G T			A A	тт	T A		
				310						320						330						340						350	,				36	5 0
TAZ				~ ~	~ "		m m	m /	~ ~			n /	r c		~ x	m ~	~ (٠,	7 7	יגי	т т	G T	т (~ m	a (· Tr	G T	та		ጥ አ	c c	AT	G C	Tr.
1 A 2	H. I	GG	1 A	370	C 1	. 1				380				Α,		390		- ^	л,		• •	400				•		410					42	
TG	A A	ТG	A C	g a	тс	C	C A	A :	r c	A C	C A	. G 2	A T	T	C A		C Z	A A	G 1	G.	A T			ГT	r	T	ΑТ			C A	c c	AT		
				430						440						450						460						470)				48	80
														_																~ ~				•
CC	CT	АА	тт	A T 490	тт	C.	АА	T		A A 500	TG	; C (C T	A.	r G	510	A	j C	ΑΊ	G	T A	520		rr	TI	Т	rı	530		J 1	A A	AC		40
																				,												↓		
М	ET	AS	N I	HIS	п	E	TY	2	LYS	; ,	VAL	I	Œ	P	HB	AS	Į.	LYS	3	↓ ALA	12	THR.	Œ	LY	נד	R.	PH	B	MET	: .	ALA		L ¹⁹	ALA
CA	r G	A A	тс		ΑΊ	C	ΤA	T	A A			' A '	rc	T '	ГТ	A A 570	C Z	A A	A C	3 C	C A	C A 580		3 C	A (A	тт	T A		G G	C A	GT		C 00
				550						560						5/0						`						370	•					,0
								_								_			_	_		~ ~				_	~	v	ŒN.	, ,	VAL	ŒT7	, 39	SER
A G	LUJ A. G.	TY T A		ALA CC					HIS CA													CYS GT						•	CEN					
				610						620						630						640						650						60
																											1							
V	AL	CY	S	THR	L	IJ	SE	R	PHI	3 2	ALA	A	RG	I	LE	AL	A	ALI	A	Œ		ALA	VZ	AL	L	IJ	VA	T ₂₂	ILE	š	ŒX	AL	A '	THR

T G T A T G C A C T C T G A G C T T T G C C G T A T T G C C G C G C T C G C T G T C C T C G T G A T C G G T G C A A C

700

710

3' Half Constructs of 200 kD Protein Gene FIGURE 18

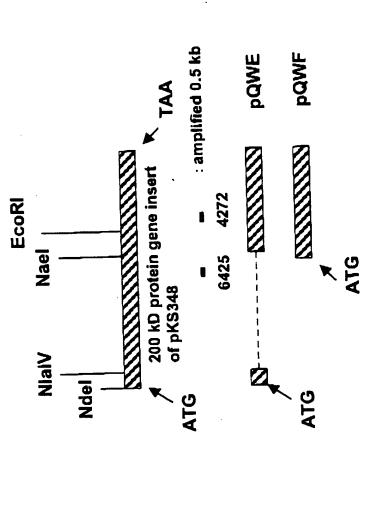


Figure 19 Construction of pQWE

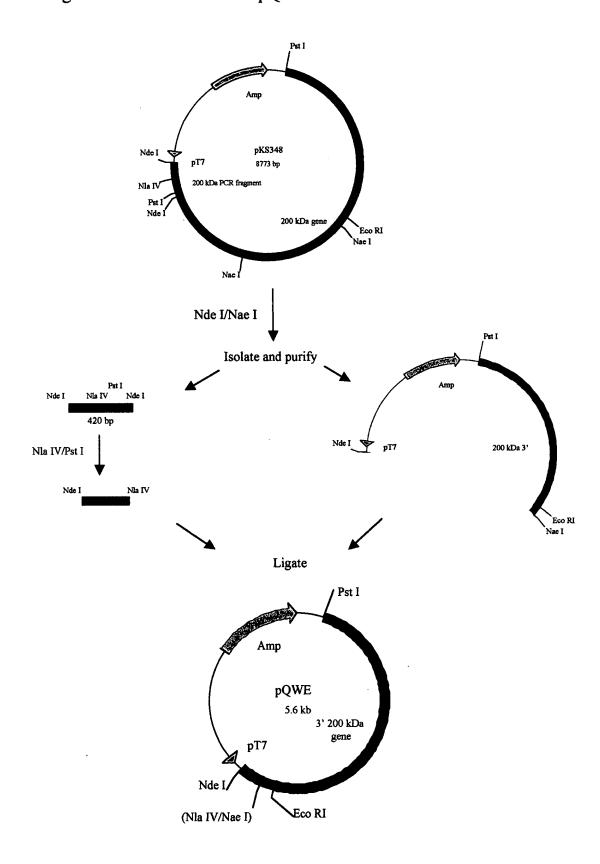


Figure 20 ' Construction of pQWF

